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Patent- og
Varemærkestyrelsen
Erhvervsministeriet

Taastrup 18 February 2000

Karin Schlichting
Head Clerk

Title: Biocompatible material with a novel functionality

Technical field of the invention

The invention is in the area of biomaterials, i.e. those materials that are used in contact with living tissue and biological fluids for prosthetic, therapeutic, storage or other applications. The working environments of any biomaterial are either biological fluids or living tissue, and the events occuring at the contacting interface play a crucial role in the overall performance of a biomaterial. Many conventional biomaterials lack the ability to properly interact with or support biological matter coming into contact with said biomaterials leading to undesired biological responses. However, these undesired response may be controlled by altering the chemical and physical properties of the surface of said biomaterials. In this respect, surface modification represents a well known strategy of providing suitable biocompatible materials. The present invention teaches a novel approach of creating biomaterial surfaces, said surfaces being capable of functionally interacting with biological material.

Background of the invention

When biological and synthetic materials interact with each other, one must contemplate that an association is formed that is normally not part of a biological environment such as e.g. the human or animal body. A biocompatible material has been defined as a material that, when interacting with biological material, does not induce an acute or chronic inflammatory response and does not prevent a proper differentiation of implant-surrounding tissues[1078] Furthermore, according to another current understanding, are biocompatible materials capable of i) controlling or guiding cell growth and tissue organization, ii) promoting or inhibiting cell-cell or cell-tissue interactions[11], iii) isolating transplanted cells from the host immune system[12], and iv) regulating production and/or secretion of cellular products.

However, many synthetic materials which are used as biomaterials are not biocompatible according to this definition, and many efforts are undertaken to find ways to improve the biocompatiblity of these materials.

One important example for the interaction of biological and synthetic materials, is the adhesion of human or animal cells to polymer substrata: Cell adhesion is known to involve various adhesive proteins, such as e.g. fibronectin (FN) and vitronectin (VN), that are adsorbed to the surface of the synthetic material and mediate a contact between said surface and adhering cells[1-5]. These interactions are furthermore mediated by specific transmembrane receptors belonging to the integrin family of cell adhesion molecules[6,7]. Adsorption of proteins from biological fluids onto a surface of a polymer is dependent on the physico-chemical properties of said polymer surface[2,8]. For example, it is well known that adhesive proteins adsorb abundantly onto hydrophobic polymer surfaces, but their adsorption, mainly driven by hydrophobic interactions, leads to conformational alterations and eventually to their deactivation and/or denaturation[4,9] (see Fig.1). These conformational alterations of the adhesive protein explain the reduced or eliminated interaction between said adhesive protein and a cell[5,10], leading to reduced polymer – cell interactions.

Furthermore, conformational alterations of proteins that adsorb to the surface of a synthetic material, as e.g. the surface of an implant or of a medical device, may also give rise to increased thrombogenicity of said material or to foreign body reactions and consecutive rejection of the implantation or medical device.

Synthetic polymers are a class of materials frequently used as biomaterials with selection criteria based on their mechanical properties, stability, and capabilities of producing predefined or desired shapes and/or morphologies. However, these materials are often not biocompatibile. For example, synthetic polymers in current use for the preparation of membranes with controlled permeability, e.g. polysulfones, polyesters or polypropylene, are often less than adequate for the

immobilization of tissue cells because the functionality of these cells cannot, due to the above described reasons, be maintained over sufficiently long periods of time.

However, the biocompatibility of any substratum may be controlled by altering the chemical and physical properties of said substratum. Surface modification represents a well known strategy of providing suitable biocompatible materials.

Hence, polymer surfaces are e.g. modified through the addition of charged side-groups to the polymer backbone[13], adsorption or covalent immobilization of biologically active proteins and peptides to the polymer[14], and the alteration of the texture or morphology of the polymeric substrate[15,16].

It is known, that surface modifcation is of particular interest when performed using e.g. a selective reaction initiated under mild conditions, such as e.g. photo-grafting[18]. Using a selective reaction, the shape of the susbtratum, including macro- or microporous structures, as well as mechanical properties, can be established and/or preserved. Examples of surface functionalizations include macroscopically homogeneous polymeric surfaces that may i) repel cells due to charge[19] or hydrophilicity/flexibility[20], or surfaces to which ii) cells may adhere via e.g. conditioning of a protein on a hydrophobic surface or via attachment or operably linkage of the protein to a peptide mimicking the binding domain of an adhesive protein[13,20].

Patterns of functionalization on a µm-scale are well suited to create patterns capable of attaching cells as well as patterns of cell-free areas[21]. Furthermore, patterns on sub-µm scale, made up e.g. by a mixture of adhesive peptides and charged groups[22], are also suited as supports for cell cultures. Photo-grafting in ~~combination with photo-lithographic techniques (ref.) is an established way of~~ achieving such patterns.

An important general class of surface modification is the attachment of macromolecules to the underlying surface. Often, will these macromolecules exhibit

hydrophilic properties and thus be solvated in an biological environment, whereas the underlying surface is e.g. of hydrophobic character. The attachment of macromolecules can be achieved through the i) physical adsorption of amphiphilic macromolecules, ii) use of self-assembled monolayers (SAM){ref.}, iii) ionical binding of charged macromolecules to surface-bound countercharges, {ref.} iv) grafting of either photo- or thermally-reactive macromolecules, {ref.} and, in the case were the underlying surface is polymeric, also through the, v) entanglement of said macromolecules into the polymer surface.{ref.} (see also Garbassi, F., Morra, M., Occhiello, E. Modification techniques, in: *Polymer surfaces - from physics to technology*, John Wiley & Sons Ltd, Chapter 6-8, Chichester (England) (1994). 221ff; Ratner, B.D. *Surface modification of polymeric biomaterials*, 1st ed., Plenum Press, New York (1997).

It is known to coat and shield surfaces of hydrophobic basis polymers with e.g. layers or chains of (attached) hydrophilic macromolecules in order to exclude biological material such as proteins and consequently also cells from coming into contact with said surface. In particular well known are the shielding properties of poly (ethylen glycol), PEG, a well known hydrophilic polymer. The protein repellent character of substrata coated with PEGs is accredited to a combination of several molecular mechanisms,{138} where consensus seems to be reached that the steric stabilization forces induced by the excluded volume of the attached macromolecules represents the dominating mechanism.{946,664} It has been observed, that the protein repelling character of PEG-coated substrata is dependent on their lateral density on a subsratum surface.{964,1022} where a correlation between amount of adsorbed protein and lateral density was observed: the higher the lateral density of attached PEGs, the lower the adsorption of proteins. According to the above, different techniques are known which describe how to prepare coated substrata, where the coats effectively shield the underlying substratum, and where said coats can also be laterally patterned (see Fig.2).

Recently, Sofia et al.[31] characterized protein (FN, cytochrome-c, and albumin) adsorption on PEG-grafted (Mw of 3.400, 10.000, and 20.000) silicon surfaces

over a range of grafting densities. Additionally, the grafted amount of PEG moieties could be measured with ESCA Protein adsorption decreases for all proteins with rising PEG grafting density and is reduced by more than 95 % for the highest PEG grafting density when compared to the unmodified silicon substrate. From measurements of the thickness of the adsorbed protein layer was deduced, that FN, having a rodlike shape, adsorbs for all PEG grafting densities "lying down" with its long axis parallel and in close contact to the surface. Thus, proteins are able to penetrate the PEG-layer to effectively adsorb to the underlying surface in between grafted chains. Sofia et al. calculated that protein adsorption started to decrease for grafting densities were grafted PEG chains began to overlap, and that protein adsorption became negligible when PEG chains were, due to the grafting density, confined to approximately half of their relaxed volume. This correlation between protein adsorption and PEG chain overlap was found for all investigated PEG molecular weights, and the calculations were based on the assumption, that grafted PEG chains exert the same radius of gyration or spatial dimension as in their solvated state.

Contact angles (CAs) are generally used to characterise the wettability of surfaces. Wettability of a surface is related to its hydrophilicity as constituated by the moities forming that surface. It is a very sensitve technique with a probing depth of approx. 5-10 Ångstrøm. CAs are determined by measuring the angle defined by the phase limits of the liquid phase at a three phase boundary (solid/liquid/vapor). The three phase boundary is generated by e.g. a vapor bubble in a liquid, where the bubble is captivated by the test surface (captive-bubble method) or a drop of liquid in vapor placed on top of the test surface (sessile-drop method). When a polar (hydrophilic) test liquid (as e.g. water) is used, hydrophilic test surfaces will generate small angle values such as 0°-90°, while hydrophobic test surfaces will generate large ~~angle values of 90°-180°. Both advancing and receding measurements of CA's are~~ used to characterize the biomaterials according to the invention.[17)]

Ellipsometry is an optical *in situ* technique for measuring i) the refractive index of a bare surface, or ii) the thickness and refractive index of a film/coat on a substratum,

both based on measuring the change in the state of polarization of light upon reflection from said substratum surface. The determined thickness and refractive index of an adsorbed layer of macromolecules can thus be converted to a value of adsorbed mass.{828} In this way it is possible to monitor on-line the adsorption of e.g. macromolecules out of solution onto a substratum surface interfacing that solution. There are detailed descriptions of the physical principles of the method{618} and the instrumental setup{874}.

Studying cell adhesion

The quality of the interactions of a synthetic material surface with biological material, i.e. the biocompatibility of said material surface, can be related to the behavior of living cells when in contact with said surface. Accordingly, criteria like the amount of adhered cells, overall cell morphology, cell migration, focal adhesion formation, extra cellular matrix (ECM) formation, and cell proliferation on the material surface are considered important when aiming to monitor and control the biocompatibility of a material surface in vitro.

The below-identified documents are believed to constitute the closest prior art in relation to the present invention. The prior art teaches how to shield a polymer from biological material, it does not address the technical problem solved by the present invention.

Sofia et al. (1998), Macromolecules 31, 5059-5070, compares different PEG type molecules, and their interaction with proteins when chemically grafted to a polymer substratum. The document does not disclose the binding of biological material in an ~~active form to the disclosed polymer material.~~

US patent no. 5,776,748 is related to a device comprising a plurality of cytophilic islands and cytophobic regions established by self-assembled monolayers exhibiting cytophilic or cytophobic endgroups. Cell-adhesion is promoted or

inhibited on the cytophilic or cytophobic regions respectively by known mechanism, as e.g. introduction of polar groups, charges, and others, and does not disclose the binding of biological material in an active form.

US patent no. 5,002,582 is related to a method of producing biomaterials having an "effective" solid surface characterized by the properties of the hydrophilic polymer and not of the solid hydrophobic surface (column 8). The claimed biomaterials do not have a contact angle that is substantially similar to that of the solid surface. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

US patent no. 4,973,493 is related to a method of producing a solid surface that is effectively shielded by a biocompatible agent. The claimed biomaterials are unlikely to have a contact angle that is substantially similar to that of the solid surface. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

US patent no. 4,722,906 is related to a method for selectively binding specific molecular target moieties covalently to a chemical moiety or substratum. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

US patent no. 5,128,170 is related to a method for manufacturing a medical device having a highly biocompatible surface. The claimed biocompatible surface does not have a contact angle that is substantially similar to that of the medical device. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

US patent no. 5,728,437 is related to an article comprising a hydrophobic surface coated with a blood compatible surface layer. The coated surface does not have a contact angle that is substantially similar to that of the hydrophobic surface. The

document does not disclose the binding of biological material in an active form to the disclosed polymer material.

US patent no. 5,380,904 is related to a method for rendering a surface biocompatible. The biocompatible surface does not have a contact angle that is substantially similar to that of the untreated surface. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

US patent no. 5,512,329 is related to methods of attaching a polymer to a surface of a substrate by application of an external stimulus. The method of claim 14 is directed to a method of modifying surface properties of a substrate. A biomaterial comprising a polymer substratum and a macromolecule and a first determinant capable of bringing a second determinant into contact with said first determinant is not disclosed. Neither does the document disclose the binding of biological material in an active form to the disclosed polymer material.

US patent no. 5,217,492 is related to a specialized means for attaching a biomolecule to a hydrophobic surface. The disclosed means for attachment is not pertinent to the present invention. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

US patent no. 5,263,992 is related to a biocompatible device comprising a solid surface and a biocompatible agent positioned sufficiently proximate to one another so as to effectively shield the solid surface. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

~~**US patent no. 5,741,881** is related to a bio-active coating that exploits a~~ hydrophilic spacer with functional end groups and capable of linking a specialized polymer with a bio-active agent. The present invention does not exploit a bifunctional linker in the form of a hydrophilic spacer as a means for attaching a

first determinant to a polymer substratum. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

WO 97/46590 is related to a material comprising a support and two layers, of which the second, outer layer is a hydrophilic polymer, said material further comprising immobilized biological material. The surface generated by coating a support with a polymeric surfactant and hydrophilic polymer does not have a contact angle that is substantially similar to that of the support. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

WO 97/18904 is related to a method for providing a hydrophobic surface with a hydrophilic coating. The surface generated by hydrophilic coating does not have a contact angle that is substantially similar to that of the hydrophobic surface. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

EP 633 031 A1 is related to a composition that is effectively capable of shielding a polymer from biological material. The shielded polymer does not have a contact angle that is substantially similar to that of the unshielded polymer. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Park and Griffith (1998), J. Biomat. Sci. Polym. Ed. 9, p. 89-110, discloses a specialized PEG-PPO-PEG copolymer scaffold capable of effectively inhibiting cell adhesion. The copolymer is useful in regulating the three dimensional organization of diverse cell types. Adhesion is achieved by covalent linkage to the polymer of a cell specific carbohydrate ligand capable of binding a particular receptor moiety. ~~The present invention is not concerned with a polymer substratum being contacted~~ with a first determinant. The cell adhesive properties of the biomaterial according to the present invention are at least partly determined by the cooperativity of a polymer substratum and a macromolecule and optionally also by a first determinant. The polymer "backbone" of the present invention is not cytophobic per

se, as is the case in the cited reference. The disclosed copolymer material is not pertinent to the present invention, and the document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Noh et al. (1998), J. Biomat. Sci. Polym. Ed. 9, p. 407-426, discloses a modification of PTFE films that substantially alters the contact angle. The disclosed biomaterials are not pertinent to the present invention, and the document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Malmsten et al. (1998), J. Coll. and Interface Science 202, p. 507-517, examines the effect of chain density on inhibition of protein adsorption. The document does not mention the properties of the bound proteins, and the document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Zhang et al. (1998), Biomaterials 19, p. 953-960, discloses silicon surfaces that are modified with a PEG film in order to reduce protein adsorption. The silicon surface does not have a contact angle that is substantially similar to that of the PEG-coated material. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Herbert et al. (1997), Chemistry and Biology 4, p. 731-737, discloses a method of differentiating the cross-linking of bioactive molecules to a surface. Biomaterials according to the present invention are not disclosed and the disclosed method is not pertinent to the present invention as photo-reactivation is acknowledged to form part of the prior art. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Wesslén et al. (1994), Biomaterials 15, p. 278-284, discloses a surface modification of a hydrophobic polymer by use of hydrophilic polymers including PEG. The modification significantly changes the contact angles (Table 1) and leads

to a reduced polypeptide adhesion. The disclosed biomaterials are not pertinent to the present invention, and the document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Bergström et al. (1992), J. Biomedical Materials Research 26, p. 779-790, discloses a polystyrene comprising densely packed and covalently bound PEG capable of effectively reducing adsorption of fibrinogen. The polystyrene does not have a contact angle that is substantially similar to that of the densely packed PEG surface. The document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Desai and Hubbell (1991), Biomaterials 12, p. 144-153, discloses an incorporation of PEG and similar water-soluble polymers onto surfaces of biomedical polymers such as e.g. PET and the like. The incorporation significantly alters the contact angle as illustrated in Table 1. The disclosed biomaterials are not pertinent to the present invention, and the document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Gombotz et al. (1991), J. Biomedical Materials Research, 25, p. 1547-1562, discloses a modification of PET surfaces with PEG. The incorporation significantly alters the contact angle as illustrated in Fig.2 and the first paragraph of the discussion. The disclosed biomaterials are not pertinent to the present invention, and the document does not disclose the binding of biological material in an active form to the disclosed polymer material.

Summary of the Invention

The present invention teaches a novel approach of creating biocompatible surfaces, said surfaces being capable of functionally interact with biological material. Said biocompatible surfaces comprise at least two components, such as a

hydrophobic substratum and a macromolecule of hydrophilic nature, which, in a cooperativity, form together the novel biocompatible surfaces.

The novel approach is based on contacting said hydrophobic substratum with a laterally patterned monomolecular layer of said hydrophilic and flexible macromolecules, exhibiting a pronounced excluded volume. The thus formed two component surface is, in respect to polarity and morphology, a molecularly heterogenous surface. Structural features of said macromolecular monolayer (as e.g. the layer thickness or its lateral density) are determined by, i) the structural features of the layer forming macromolecules (as e.g. their molecular weight or their molecular architecture) and, ii) the method of creating said monomolecular layer (as e.g. by physi- or chemisorbing, or by chemically binding said macromolecules). The structural features of the layer forming macromolecule(s) is in turn determined by synthesis.

Amount and conformation and thus also biological activity of biological material (as e.g. polypeptides) which contact the novel biocompatible surface, is determined and maintained by the cooperative action of the underlying hydrophobic substratum and the macromolecular layer. In this way it becomes possible to maintain and control biological interactions between said contacted polypeptides and other biological compounds as e.g. cells, antibodies and the like. Consequently, the present invention aims to reduce and/or eliminate the deactivation and/or denaturation associated with the contacting of polypeptides and/or other biological material to a hydrophobic substratum surface.

In a preferred hypothesis, solvated polypeptides penetrate the laterally patterned monolayer of macromolecules to effectively adsorb inbetween said macromolecules to the underlying hydrophobic surface. Said polypeptides must, in ~~order to penetrate the monolayer of macromolecules, deform said self-assembled~~ macromolecules to some degree, inducing a lateral pressure acting between said macromolecules and penetrated polypeptides, but also between said macromolecules themselves (see also Fig.3). This lateral pressure has its origin in the unfavorable loss in conformational entropy of said bound macromolecules

related to the spatial deformation of said macromolecules. The lateral pressure will therefore increase as the amount of penetrated polypeptides increases.

Consequently, the amount of adorbed polypeptides will, according to the hypothesis, continue to increase until an energetically favorable balance is attained between, i) the unfavorable induced lateral pressure, and ii) the favoreable adsorption of said polypeptides to the underlying hydrophobic surface. In other words, polypeptides will continue to penetrate the macromolecular layer to effectively adsorb to the underlying hydrophobic surface until the hereby induced lateral pressure in that layer will effectively repell any other polypeptides from that layer.

According to this hypothesis, experience polypeptides adsorbed inbetween said self-assembled macromolecular layer a lateral pressure originating from surrounding and deformed macromolecules. The lateral pressure acting upon adsorbed polypeptides, will effectively protect said polypeptides from unfolding/denaturation, and stabilize said polypeptides in an active conformation, yielding adsorbed but biologically active polypeptides.

The invention thus solves the problem of how to provide - by simple and inexpensive methods - general surface design principles and modification methods in order to enable the control of attachment, spreading, growth and tissue formation of cells on surfaces, as these depend on biologically active polypeptides present at a surface. These novell biocompatible surfaces may thus be used as cell-culture dishes, bioreactors, implants, and the like, without the need of extensive development of new polymers and biocompatibility screening.

It is therefore contemplated, that the present invention provides means to create biocompatible surfaces suitable for use in emerging technologies such as e.g. the construction and application of novel surface architectures of biomaterials with innovative functionalities. Accordingly, the invention is useful in the manufacture of surface architectures for use in biohybrid organs, such as e.g. a bioartificial

pancreas, liver or kidney. The invention will enable the use of improved membranes for ensuring spatial separation of e.g. xenogenic and/or allogenic cells from the host immune system[12].

Modifying membranes with said macromolecular layers comprising hydrophilic macromolecules such as e.g. poly(ethylene glycol) (PEG) may according to the present invention reduce the amount of adsorption of proteins on the plane of the membrane[24] and at the same time improve the conformational/functional state/form of adsorbed proteins such as FN and other attachment proteins.

The present invention also contemplates providing arrays for culturing "sensual" cells such as e.g. nerve, olfactorial, retina, and similar cells. Culturing of sensual cells requires a spatially resolved reception of signals that must be organized in a highly complex and specific manner. The signals generated by those cells must be transmitted to a non-biological support in a time resolved and location dependent manner. Photolitographical techniques involving e.g. the immobilization of PEG spacers and bio-specific ligands may be used to contribute to the structuring and/or functionalization of solid supports in a highly specific way. It is envisaged that such structures may eventually be used e.g. as sensors or biohybrid organs.

Cells capable of being immobilized onto the biomaterials according to the invention are preferably, but not limited to, cells the function of which comprise i) controlled delivery of biologically active substances, such as e.g. hormones, ii) production of predetermined proteins and polypeptides derivable therefrom, such as e.g. antibodies, growth factors, matrix factors, and the like, or iii) the conversion of metabolites, preferably toxic or cytostatic metabolites. Examples for such types of cells are e.g. Langerhans islets cells, hybridoma cells, chondrocytes, and hepatocytes.

It is contemplated that the invention is useful in the organization of cells in organs and tissues. Such an organization involves a controlled co-operation of different types of cells that are connected, on a micrometer scale, through a local and highly organized network of different cell types. It is contemplated that the present

invention will allow photolitographical techniques to be applied in the immobilization of macromolecules with distinct functionalities and biogenic ligands. The biomaterials thus generated are capable of immobilizing different types of cells in a controlled and/or spatially structured manner so as to make them available for a controlled co-operation.

It is also contemplated to obtain an organization of cells in organs and tissue-like structures by stochastically distributed macromolecules (e.g. with and without specific functionalities, such as, e.g. amine groups, either itself or for subsequent immobilization of biological or biomimetic receptors) on a solid support, and subsequently use a second ligand (e.g. another macromolecules with a different functionality such as e.g. a functionality exerted by e.g. a different chain length) in the formation of clusters of different sizes (e.g. clusters with a different length with regard to an axis, e.g. the z-axis) and/or functionality. In this way, the invention makes it possible to obtain a patterning of a given substrate in three dimensions. This may eventually offer the possibility of providing structured surfaces for the immobilization of e.g. a single type of cells, or e.g. co-culture different cells by binding ligands that are selective for specific cell surface receptors, such as integrins, growth factor receptors and the like.

The novel and innovative applications described herein above cannot be realized with the state of the art means currently available, because there exists a profound lack of useful design principles and suitable methods for surface modification. Also, the state of the art methods are not readily applicable to fine-tune the surface structure and/or biocompatibility of known polymeric biomaterials. The invention described herein represents a significant improvement of the state of the art techniques and potentially enables the creation of novel biocompatible materials and cell-based technologies.

Acording to one preferred aspect of the present invention, the biocompatible surface has a contact angle that is substantially identical to the contact angle of the underlying hydrophobic substratum of said surface. The biocompatible surface

according to the invention differs from prior art hydrophobic substrata that are coated with a hydrophilic layer, as such prior art surfaces have a contact angle that is significantly different from that of the basis substratum. Consequently, the conversion of a hydrophobic substratum having a predetermined contact angle into a biocompatible surface having essentially the same contact angle is believed to be novel. The biocompatible surface may further comprise a first determinant, e.g. an adhesion polypeptide, capable of bringing a second determinant, e.g. a biological cell, into reactive contact with said first determinant.

In yet another aspect of the invention, the biocompatible surface is capable of interacting with at least one first determinant (e.g. a polypeptide) and maintain said first determinant in an active form, preferably an active conformation. The presence of said first determinant in its functional form and/or active conformation results in an improved first determinant-mediated contact between said biocompatibel surface comprising said first determinant and e.g. a second determinant such as a cell capable of contacting said first determinant and preferably forming a stable association therewith.

In one particular aspect the invention pertains to a material having a first contact angle and comprising a substratum having a second contact angle, said substratum being contacted by a macromolecule, wherein the relation between said first and second contact angle as defined by the ratio between

i) the difference between said second contact angle, when no macromolecule is present, and said first contact angle, and

ii) the difference between said second contact angle, when no macromolecule is present, and the contact angle of said substratum, when said substratum is saturated by said macromolecules as ~~defined herein,~~

is more than -0.6 and less than 0.6.

In another aspect the invention pertains to a material having a first contact angle and comprising a substratum having a second contact angle, said substratum

being contacted by a plurality of soluble substances capable of forming a self-assembled monolayer comprising a macromolecule and having a third contact angle, wherein the relation between said contact angles as defined by the ratio between

i) the difference between the third contact angle of said monolayer, when no macromolecule is present, and said first contact angle, and

ii) the difference between the third contact angle of said monolayer, when no macromolecule is present, and the contact angle of said self-assembled monolayer, when said monolayer is saturated by said macromolecules as defined herein,

is more than -0.6 and less than 0.6.

The described properties of a biocompatible surface according to the invention comprising said hydrophobic substratum and said hydrophilic macromolecule are useful, as a first determinant can adhere to and remain associated with said surface in a functional conformation or a biologically active form or conformation. The properties of said surface comprising said substratum and said macromolecule and said first determinant are also useful, as a second determinant can adhere to and remain associated in a functional or active form or conformation, preferably a biologically active form or conformation, with said first determinant and consequently with the surface.

Detailed description of the invention

The follwoing definitions are being used to illustrate the present invention.

Definitions

Active conformation: protein in a conformation, where it has its normal biological activity in a native host organism.

Active form: protein or biological material in a form, where it has the same function as when said protein or biological material is present in native host or native environment.

Adsorption: the taking up of molecules from a gas or liquid on the surface of another substance such as a substratum.

Advancing contact angle: contact angle when the liquid front is caused to advance over said solid.

Amphiphil: substance containing both polar, water-soluble and nonpolar, water-insoluble groups.

Arrays for culture of "sensual" cells: Solid or semi-solid supports with ordered structures for the attachment of sensual cells, such as retina cells.

Biocompatible material: Material that, when interacting with biological material, does not induce an acute or chronic inflammatory response and does not prevent a proper differentiation of implant-surrounding tissues.

Biologically active form: see active form.

Biologically active conformation: see active conformation.

Biological material: Any material derived from a living entity including plants, animals or a living part thereof, such as an organ or cell. The preferred biological system is a mammalian system, preferably a human system.

Biomaterial: A material interfacing with biological systems to e.g. evaluate, treat, augment or replace any tissue, organ or function of the body.

Biogenic ligand: Any ligand of biological origin, such as carbohydrates, proteins or parts thereof such as e.g. oligopeptides, including any combination and/or derivatives thereof.

Biohybrid organ: A device comprising a combination of a biomaterial and a biological material in an active form, such as e.g. specific organ cells.

Cell differentiation: Process by which a precursor cell becomes a distinct specialised cell type.

Conformational alterations: Change in the overall three dimensional form of a material, usually a biological material.

Conformational entropy: The entropy of a macromolecule as determined by the amount of possible conformations that the macromolecule may attain.

Conjugate: Plurality of functional molecules chemically joined together.

Contact angle (CA): Angle (θ) represented by the limits of the liquid phase at a three phase boundary between a solid or semi-solid surface, a liquid and the saturated vapor of said liquid. Different methods are applied to generate a three phase boundary, as e.g. the captive bubble method, where a bubble of staturated vapor of the used test liquid is captivated by the test surface.

Deactivation: Alteration of an active form or conformation to a less active form or conformation.

~~**Density:** Mass per volume (concentration) or per area (lateral density).~~

End group: Distal part of a macromolecule.

Excluded volume: Interaction between segments of solvated macromolecules or polymer chain(s) that are moving to occupy the same space.

Extracellular matrix (ECM): Meshwork synthesized by cells and composed of adhesive proteins such as glycoproteins, laminin, fibronectin, interconnected collagen fibrils, hyaluronate and proteoglycans as structural and functional support of tissue cells.

Film: Synthetic material in the form of long, thin sheets.

Flexible: Capable of attaining many conformations, in contrast to rigid.

Flux: Measure of the flow of some quantity per unit area per unit time

Functionalization: Chemical derivatization changing structure, properties and/or function.

Grafting: Attaching at least one macromolecule comprising equal or different molecular units to a substratum through a chemical bond.

Head group: Proximal group, the group forming the link between a macromolecule and a substratum.

Hydrophilic polymer: Any polymer with a high surface energy where droplets of water spread readily.

Hydrophobic polymer: Any polymer with low surface energy where water forms prominent droplets on the surface.

Improved contact: Enhanced attachment and spreading of cells upon contact with non-biological supports.

Interface: Area or surface that represents the boundary between two separate phases of a chemical or physical process.

Ionic bond: Bond held together by coulombic interactions between differently charged moieties.

Latent: Present but not (yet) active.

Laterally structured monolayer: Monolayer formed of macromolecules interacting with neighboring molecules due to their inherent excluded volume, to sponateously form a relatively ordered array of macromolecules, said monolayer is not crystalline and characterised by a water content of at least 50 percent.

Layer density: Mass per area (2D concentration).

Linker: Connects two moieties or groups or molecules with each other.

Macromolecule: Any molecule having a molecular weight higher than 400 Dalton.

Membrane: Barrier between two phases and allowing transport via sorption/diffusion and/or through pores.

Permeability: Measure of the capability of a membrane to allow transport through said membrane.

Photo: Physical stimulus, here to initiate a chemical reaction.

Photo-reactive polymer: Polymer comprising one or more latently reactive groups.

Polymer: Molecule formed by the union of at least five identical monomers

Pretreatment: The addition of functional groups to a substratum.

Receding contact angle: Contact angle when the liquid front is caused to recede over said solid.

Refractive index: Ratio of the phase velocity of electromagnetic radiation in a vacuum (or air) to that in a transparent medium.

Rigid: Essentially non-flexible.

Saturated monolayer: Saturation is achieved when no significant change of the contact angle of said monolayer contacted by a plurality of macromolecules is observed, when said monolayer is being contacted by more macromolecules.

Saturated substratum: Saturation is achieved when no significant change of the contact angle of said substratum contacted by a plurality of macromolecules is observed, when said substratum is being contacted by more macromolecules.

Self-assembled monolayer: Monolayer formed on a substratum and comprising self-assembled (stacked or crystallized) components comprising a headgroup, said headgoup interacting favorably with the substratum, and an endgroup, said endgroup being orientated towards the solution. Said monolayer is characterized by a crystalline, highly ordered structure and a very low water content or substantially no water content.

Solvated: Molecule or material being in solution.

Synthetic material: Any material that is not of biological origin.

Substratum: Any chemical moiety to which macromolecules are capable of attaching.

Surface: Outer part of an object, here the biomaterial or its precursor.

The present invention teaches a new way of controlling cell adhesion and biocompatibility of polymer substratum surfaces associated therewith. The novel approach is based on a structuring of a hydrophobic substratum surface, preferably a hydrophobic polymer substratum, with a layer of macromolecules, preferably a monomolecular layer of flexible macromolecules, more preferably a monolayer of laterally patterened macromolecules contacted with said surface of said hydrophobic polymer substratum.

The response of essentially biological systems to the designed surfaces according to the invention is different from the response of such systems to the polymers of the prior art. Studies with human fibroblasts – i.e. a biological material well accepted as a general cellular model for tissue-biomaterial interaction - have been carried out in order to evaluate the ability of fibroblasts to attach, spread and proliferate on various surfaces that had been modified according to the invention. The production of an extracellular matrix is one of several key functions of fibroblasts and generally a characteristic feature of cells of the connective tissue type. Consequently, the ability of fibroblasts to attach to biomaterials according to the invention has been studied by microscopical investigations of extracellular matrix formation i) within the first hours of cell attachment, by means of fluorescently labeled FN, and ii) following long-term culture through direct detection of the synthesized FN matrix.

The studies revealed an improved cellular functionality as a function of e.g. the molecular weight of the macromolecules attached to the polymer substratum, and the degree of surface functionalization. The results were measured by typical biocompatibility parameters such as e.g. cell adhesion and morphology, formation of focal adhesions points, and the formation of an extracellular matrix. All of the above is understood to contribute to the observed improved cellular functionality as defined herein above. In other words, the results clearly showed that polymer

substratum surfaces modified according to the invention has a superior functionality. The functionality is superiour when compared to both the original, unmodified polymer, and to the fully modified or "coated" surfaces of the prior art that are characterised by a comparatively high degree of surface functionalization.

The results described herein are strong indications that it is possible to further optimise the relationship that exists between adsorption of essentially biological material, the state, or conformation, or biologically active form of said adsorbed material, and the cellular behavior or functionality resulting from said adsorption.

Consequently, the invention makes it possible to determine empirically one or more optima of cellular functionality by means of a rational design approach that is readily controllable by any suitable state of the art physico-chemical surface analysis. Hence, the present invention achieves its objective by significantly improving state of the art methods of providing biomaterials, since the response of adsorbed cells and their biocompatibility can now be predetermined or at least designed quickly and economically by well-defined and readily adjustable state of the art physico-chemical and bioengineering parameters.

Materials that are capable of being processed according to the invention are those with at least suitable, if not superior physico-chemical properties for any given application, such as e.g. suitable or superior properties like transparency, refraction index, elctrical conductivity, thermal stability, hydrolytic resistance, or membrane forming properties (ranging from, e.g. cell-culture dishes to membranes), but are currently less than adequate, if not entirely useless, for the attachment, growth and function of cells because of their undesirable physico-chemical surface properties.

The surface structures of the biomaterials to be processed in accordance with the invention may be porous structures with a stochastic or predetermined or controlled permeability (e.g. micro- or macro-porous flat-sheet or hollow-fiber membranes) that may be built up as a temporary or permanent support of cells described herein immediately below.

The two-step modification technique disclosed herein preferably generates a covalently bound, patterned molecular monolayer. The structure or functionality of the layer may be designed or predetermined by synthesis of macromolecule conjugates and then in a first adsorptive step according to any given set of particular circumstances. By covalent grafting, a stable attachment (i.e. grafting) to the underlying polymeric material (basis polymer) is readily achieved. The control of the "design parameters" such as e.g. molecular structure of the amphiphilic macromolecule, the concentration and/or solvency of said macromolecule can be left to a person skilled in the art of manufacturing complex polymers.

Molecular weight and/or size of the amphiphil determines at least to some extent the molar density (i.e. macromolecules per surface area). An increased interaction between the amphiphilic macromolecule and the polymer substratum is likely to lead to an increased layer density. Likewise, a high concentration of amphiphilic macromolecules in the first step (see Fig.), or a decreased solvency of said amphiphilic macromolecules will also contribute to an increased layer density. Changes in solvency may be attainable through variations in e.g. salt concentration, pH, temperature or polarity of the solvent. Application of the amphiphiles by spray-coating and subsequent drying followed by UV/Vis irradiation can be alternative technologies. The person skilled in the art is familiar with the physical chemistry of polymers and macromolecules required in order to attain an altered layer density.

It is understood that when the biomaterial is a film, the polymer substratum is substantially inpenetratable to water, whereas the polymer substratum is porous, when the biocompatible material is a membrane.

The created lateral layer structure according to the invention is characterized by the amphiphil nature of the macromolecule and amphiphil-amphiphil intermolecular and intramolecular interactions. Strong repulsive interactions between the amphiphiles

due to their inherent large excluded volumes lead to discretely adsorbed molecules capable of forming a laterally "self-assembled" structure.

According to one preferred embodiment of the invention, surface functionalization is mediated by well-defined photo-reactive conjugates of hydrophilic, flexible macromolecules comprising a modular composition of building blocks. In one particularly preferred embodiment said modular composition comprises:

(Latent-reactive head-group)-(guiding-group)-(main body)-(functional end-group)

The invention aims to provide a substratum surface with desired physical characteristics and comprises the steps of contacting the substratum with a composition comprising a plurality of macromolecules possessing desired physical characteristics. The macromolecules each comprise covalently bonded, optionally via a linker group, to their main body, a latent-reactive head-group, and optionally also a guiding group, and a functional end-group. The latent-reactive head-group is capable of providing one or more active species such as free radicals in response to external stimulation to covalently bind the macromolecules to the substratum, through the residues of the latent-reactive head-group.

The macromolecule is spatially oriented so as to enable one or more of its latent-reactive groups to come into covalent bonding proximity with the substratum surface, and the method according to the present invention includes the further step of activating the latent-reactive groups by applying external stimulation to covalently bond the macromolecule to the substratum. The external stimulation that is employed is preferably electromagnetic radiation, and more preferably the radiation is in the ultraviolet, visible or infra-red regions of the electromagnetic spectrum, since the layer structure established by "self-assembly" is not disturbed by this kind of radiation, and the polymer substratum is left at least substantially intact. The degree of conversion is selectable by e.g. UV/Vis dose, and typically 100% conversion will be attempted. The response to the activation step of the method can be tuned by selecting different latent-reactive groups. Also, the

reactivity of the photo-chemically generated reactive species can be selected in accordance to the structure of the polymer substratum. Thus, it is well known that e.g. aryl nitrenes from aryl azides will react via insertion reactions with all polymers having -NH, -OH or -CH groups.

The latent-reactive head-group of a macromolecule employed in the invention may comprise one or more covalently bonded latent-reactive groups. The latent-reactive groups, as defined herein, are groups which respond to specific applied external stimuli to undergo an active species generation resulting in covalent bonding to an adjacent support surface. Latent-reactive groups are those groups of atoms in a molecule which retain their covalent bonds unchanged under conditions of storage but which, upon activation, form covalent bonds with other molecules. The latent-reactive groups generate active species such as free radicals, nitrenes, carbenes, and excited states of ketones upon absorption of external electromagnetic or kinetic (thermal) energy. Latent-reactive groups may be chosen to be responsive to various portions of the electromagnetic spectrum, and latent-reactive groups that are responsive to ultraviolet, visible or infrared portions of the spectrum are preferred.

The azides constitute a preferred class of latent-reactive groups and include arylazides such as phenyl azide, 4-azido benzoic acid, and 4-fluoro-3-nitrophenyl azide, acyl azides such as benzoyl azide and p-methylbenzoyl azide, azido formates such as ethyl azidoformate, phenyl azidoformate, sulfonyl azides such as benzenesulfonyl azide, and phosphoryl azides such as diphenyl phosphoryl azide and diethyl phosphoryl azide. Diazo compounds constitute another class of latent reactive groups and include diazoalkanes ($-CHN_2$) such as diazomethane and diphenyldiazomethane diazoketones such as diazoacetophenone and 1-trifluoromethyl-1-diazo-2-pentanone, diazoacetates such as t-butyl diazoacetate and phenyl diazoacetate, and beta-keto-alpha-diazoacetates such as t-butyl alpha diazoacetoacetate. Other latent-reactive groups include the aliphatic azo compounds such as azobisisobutyronitrile, the diazirines such as 3-trifluoromethyl-3-phenyldiazirine, the ketenes (-CH=C=O) such as ketene and diphenylketene and

photoactivatable ketones such as benzophenone and acetophenone. Peroxy compounds are contemplated as another class of latent-reactive groups and include dialkyl peroxides such as di-t-butyl peroxide and dicyclohexyl peroxide and diacyl peroxides such as dibenzoyl peroxide and diacetyl peroxide and peroxyesters such as ethyl peroxybenzoate.

Upon activation of the latent-reactive groups to cause covalent bond formation to the surfaces to which macromolecules are to be attached, the macromolecules are covalently attached to the surfaces by means of residues of the latent reactive groups.

As will be noted from the foregoing disclosure, photoreactive groups are for the most part aromatic and are hence generally hydrophobic rather than hydrophilic in nature. The presence of a comparatively hydrophobic reactive head-group such as an aromatic photoreactive group, appears to be causing the macromolecule to orient itself in an aqueous solution with respect to a hydrophobic substratum surface such that the comparatively hydrophobic reactive head-group is preferentially carried near the support surface while the remainder of the macromolecule, i.e. the main body and the functional end-group, is generally orientated away from the hydrophobic substratum surface. It is known that this feature enables macromolecules to be covalently bonded densely to a comparatively hydrophobic support substratum surface, and this in turn contributes to the formation of a biocompatible substratum surface as defined above.

According to the above, the amphiphilic character and thus orientation and achieved grafting density of macromolecules to a substratum surface can be increased by incorporating a hydrophobic guiding-group into the macromolecule. The guiding-group is a bifunctional group that is positioned, preferably by means of a linker group, between the latent-reactive head-group and the remainder of the macromolecule, i.e. the main body and the functional end-group. The guiding-group is hydrophobic for the purpose of enhancing the preferential orientation of the latent-reactive head-group of the macromolecule into bonding proximity of the

substratum surface and for the purpose of increasing the amphiphilic character of the macromolecule in order to increase the achieved grafting density. Preferred classes of guiding groups are aliphatic, linear or weakly branched groups or cyclic aliphatic groups, both preferably with from 6 to 18 carbon atoms, or combinations thereof, as well as mono- or polycyclic aromatic groups, or their combinations with the above-mentioned aliphatic groups.

The main body of the macromolecule is preferably hydrophilic, uncoiling in an aqueous environment and thus exhibiting an excluded volume. It may be a polymer of natural or synthetic origin. Such polymers include oligomers, homopolymers and copolymers resulting from addition or condensation polymerization, and natural polymers including oligosaccharides, polysaccharides, oligosaccharides, and polypeptides or a part thereof, suc as an extended oligopeptide. The polymer forming the main body may comprise several distinct polymer types, as prepared by terminal or side chain grafting, including cellulose-based products such as hydroxyethyl cellulose, hydroxypropyl cellulose, carboxymethyl cellulose, cellulose acetate and cellulose butyrate, acrylics such as those polymerized from hydroxyethyl acrylate, hydroxyethyl methacrylate, glyceryl acrylate, glyceryl methacrylate, acrylic acid, methacrylic acid, acrylamide and methacrylamide, vinyls such as polyvinyl pyrrolidone and polyvinyl alcohol, nylons such as polycaprolactam, polylauryl lactam, polyhexamethylene adipamide and polyhexamethylene dodecanediamide; polyurethanes, polylactic acids, linear polysaccharides such as amylose, dextran, chitosan, and hyaluronic acid, and branched polysaccharides such as amylopectin, hyaluronic acid and hemi-celluloses.

The macromolecules themselves preferably have molecular weights of at least about 500 Da, most preferably of about 10.000 Da, and are hydrophilic in nature, and soluble in water to the extent of at least approximately 0.5 % by weight at 25°C.

In a preferred embodiment the main body comprises repeating units as e.g. ethoxy ($-CH_2-CH_2-O-$) or isopropoxy ($-CH_2-CH(CH_3)-O-$) groups, and of these poly(ethylene glycol) is most preferred.

Functional endgroups include all chemical moieties that can be used to link permanently or reversibly other biological or synthetic molecules or cells, viruses and the like via the polymeric main body to a surface, such as hydroxy, amino, carboxyl, sulphonic acid, activated esters, or epoxy groups as well as charged or chelating functionalities.

Additionally, the functional end-group may be chosen from a wide variety of compounds or fragments thereof which will render the modified substratum generally or specifically "biophilic" as those terms are defined below. Generally biophilic functional end-groups are those that would generally promote the binding, adherence, or adsorption of biological materials such as, for example, intact cells, fractionated cells, cellular organelles, proteins, lipids, polysaccharides, simple carbohydrates, complex carbohydrates, and/or nucleic acids. Generally biophilic functional end-groups include hydrophobic groups or alkyl groups with charged moieties such as $-COO^-$, $-PO_3H^-$ or 2-imidazolo groups, and compounds or fragments of compounds such as extracellular matrix proteins, fibronectin, collagen, laminin, serum albumin, polygalactose, sialic acid, and various lectin binding sugars. Specifically biophilic functional end-groups are those that selectively or preferentially bind, adhere or adsorb a specific type or types of biological material so as, for example, to identify or isolate the specific material from a mixture of materials. Specific biophilic materials include antibodies or fragments of antibodies and their antigens, cell surface receptors and their ligands, nucleic acid sequences and many others that are known to those of ordinary skill in the art. The choice of an appropriate biophilic functional end-group depends on considerations of the biological material sought to be bound, the affinity of the binding required, availability, facility of ease, and cost. Such a choice is within the knowledge, ability and discretion of one of ordinary skill in the art.

For the preparation of biodegradable coatings or coatings that may be degraded under predefined environmental conditions, it is desirable to incorporate in the macromolecule a moiety that allows either enzymatic or chemical hydrolysis of the coating. Suitable ingredients include amino acids such as alanine, valine, leucine, proline, methionine, aspartic acid, threonine, serine, glutamic acid, glycine, cysteine, phenylalanine, lysine, histidine, argine, and aminobutyric acid. Alternatively, hydrolytically unstable ester bonds can be applied as well. All these moieties are typically part of a linker group, when such a group is present, but may also be incorporated into the main-body or the guiding-group of the macromolecule.

The lateral density of the monolayer of macromolecules according to the invention is adjustable by e.g. i) modification of the amount and/or concentration of macromolecules in solution during "self-assembly", or ii) the use of mixtures of macromolecules, said macromolecules comprising varying building blocks as e.g. different molecular weights, or variations in other structural features of the macromolecule (e.g. branched vs. unbranched), or iii) adjustable by appropriately choosing solution conditions during an adsorptive application of said macromolecules, as e.g. the solvency, the ionic strength, the temperature or the pH. The process of photochemical grafting does neither disturb this "self-assembled" pattern, nor does it result in any substantial degradation of the underlying surface of the polymer substratum.

The substratum comprises a definable surface such as the tangible surface of film or a membrane, or the surface of a contact lens or surgical implant, or the surface provided by small particles in an emulsion or other suspension or as a powder, or as the surface of a soft gel. The invention provides the particular advantage of providing means by which non-pretreated definable (e.g., solid) surfaces may simply and rapidly be provided with covalently bonded macromolecular coatings in a simple, rapid and hence economical manner.

Preferred embodiments of the invention are described herein below. The material according to the invention may comprise soluble substance in the form of

molecules capable of forming a self-assembled monolayer. Also, the substratum may be pretreated or modified, preferably as the result of said substratum being contacted by and/or operably linked to a charged group or a hydrophilic compound.

The contact angle of said material is preferably an advancing contact angle or a receding contact angle. In one embodiment, the advancing contact angle is in the range of from 50 degrees to 140 degrees, preferably in the range of from 60 degrees to 125 degrees, such as in the range of from 70 degrees to 120 degrees, for example in the range of from 75 degrees to 110 degrees, such as in the range of from 80 degrees to 100 degrees.

The contact angle of said material may also be the receding contact angle, in which casethe contact angle is in the range of from 30 degrees to 120 degrees, preferably in the range of from 40 degrees to 110 degrees, such as in the range of from 50 degrees to 100 degrees, for example in the range of from 60 degrees to 90 degrees, such as in the range of from 70 degrees to 80 degrees.

The ratio between the difference between said second contact angle, when no macromolecule is present, and said first contact angle, and the difference between said second contact angle, when no macromolecule is present, and the contact angle of said substratum, when said substratum is saturated by said macromolecules as defined herein, is more than -0.6 and less than 0.6, and preferably in the range of from 0 to less than 0.50, such as less than 0.40, for example less than 0.30, such as less than 0.25, for example less than 0.20, such as less than 0.15, for example less than 0.10, such as less than 0.05.

When the contact angle is the receding contact angle the ratio is preferably less than 0.40.

The ratio between the difference between the third contact angle of said monolayer, when no macromolecule is present, and said first contact angle, and the difference between the third contact angle of said monolayer, when no

macromolecule is present, and the contact angle of said self-assembled monolayer, when said monolayer is saturated by said macromolecules as defined herein, is more than -0.6 and less than 0.6, and preferably in the range of from 0 to less than 0.50, such as less than 0.40, for example less than 0.30, such as less than 0.25, for example less than 0.20, such as less than 0.15, for example less than 0.10, such as less than 0.05.

In one particularly preferred embodiment there is provided a material which, when contacted by a first determinant comprising a compound selected from the group consisting of a polypeptide, or part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof, is capable of maintaining said compound in a biologically active form. More preferably the compound is a polypeptide or part thereof.

There is also proided a material further comprising said first determinant comprising said compound, wherein said first determinant is maintained in a biologically active form when contacted by said substratum and/or said macromolecule. The biologically active form is preferably an essentially biologically active conformation. The biologically active form or conformation is preferably maintained and/or improved and/or stabilized by means of the cooperactivity of said substratum and said macromolecule. The biologically active form or confirmation is preferably maintained and/or improved and/or stabilized when contacted by said substratum and said macromolecule. The material according to the invention is preferably biocompatible.

There is also provided a material according to the invention, wherein the weight increase per area unit arising from the part of the macromolecule essentially consisting of poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO) is less than 2.0×10^{-22} grams (g) per square nanometer (nm^2), such as less than 1.0×10^{-22} grams (g) per square nanometer (nm^2), for example less than 0.8×10^{-22} grams (g) per square nanometer (nm^2), such as less than 0.5×10^{-22} grams (g) per square

nanometer (nm^2), for example less than 0.3×10^{-22} grams (g) per square nanometer (nm^2).

Thee is also provided a material wherein the substratum is contacted by a plurality of soluble compounds capable of forming a layer of self-assembled macromolecules, preferably n-alkane chains preferably containing from 8 to 24 carbons. The macromolecule according to the invention can be characterised by an excluded volume.

The substratum preferably comprises a hydrophobic polymer and in one embodiment the substratum is at least substantially flexible and/or a film. However, the substratum may also be essentially rigid or at least substantially non-flexible. In this case, the substratum may comprise a crystalline structure capable of supporting a self-assembled monolayer such as gold, silicon oxide, and similar crystalline structures and/or structures that are smooth on a nanometer scale.

The macromolecule according to the invention comprises a hydrophilic polymer or an amphiphilic polymer. The macromolecule preferably has a molecular weight (MW) of more than 400 Dalton (Da), such as a molecular weight (MW) of more than 1.000 Dalton (Da), for example a molecular weight (MW) of more than 5.000 Dalton (kDa), such as a molecular weight (MW) of more than 10.000 Dalton (Da), for example a molecular weight (MW) of more than 50.000 Dalton (Da), such as a molecular weight (MW) of more than 100.000 Dalton (Da).

The macromolecule according to the invention is preferably a conjugate comprising a head group, a guiding group, a linker group, a polymer chain or a main body, and a functional end group.

The head group is capable of forming a chemical bond, such as a ionic bond, and may adsorb to the substratum or be entangled into or with the substratum. The head group may also be capable of forming a self-assembled monolayer.

A preferred guiding group is a bifunctional group comprising an aliphatic, linear or weakly branhed group.

A preferred linker group is capable of being enzymatically or chemically hydrolysed, it may be hydrolytically unstable, or it may be essentially stable against cleavage under practical circumstances.

The polymer chain or main body is preferably hydrophilic, uncoiling in an aqueous environment and exhibiting an excluded volume.

The functional end group is capable of linking permanently or reversibly other biological or synthetic molecules or materials.

A first determinant as defined herein comprises a biologically active compound comprising a polypeptide, or a part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof. The biologically active compound is preferably selected from the group consisting of membrane associated and/or extracellular matrix polypeptides natively produced by a microbial cell, a plant cell or a mammalian cell. The biologically active compound in another embodiment is selected from the group consisting of a polypeptide, an antibody, a polyclonal antibody, a monoclonal antobody, an immunogenic determinant, an antigenic determinant, a receptor, a receptor binding protein, an interleukine, a cytokine, a cellular differentiation factor, a cellular growth factor, and an antagonist to a receptor.

The biologically active compound may also be a synthetic polypeptide, or part thereof, capable of contacting said substratum and/or said macromolecule. Preferably the biologically active compound is an adhesion polypeptide, preferably fibronectin or vitronectin.

The biologically active compound preferably results in an improved contact between said material and a biological entity, such as a biological cell or a virus, or

part thereof, including a polypeptide, or a part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof.

In one particularly preferred embodiment the material according to the invention further comprises a second determinant as defined herein. The second determinant comprises a biological entity, such as a biological cell or a virus, or part thereof, including a polypeptide, or a part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof.

The biological entity is preferably also selected from the group consisting of a polypeptide, an antibody, a polyclonal antibody, a monoclonal antobody, an immunogenic determinant, an antigenic determinant, a receptor, a receptor binding protein, an interleukine, a cytokine, a differentiation factor, a growth factor, and an antagonist to the receptor. The biological cell, or part thereof, is preferably a mammalian cell, including a human cell and an animal cell, a plant cell, a microbial cell, including a eukaryotic microbial cell, including a yeast and a fungus, and a prokaryotic microbial cell including a bacteria.

The second determinant may also be a mammalian virus, including a human virus and an animal virus, a plant virus, a microbial virus, including a eukaryotic microbial virus, including a yeast virus and a fungal virus, and a prokaryotic microbial virus including a bacteriophage.

In one embodiment the substratum is porous and preferably a membrane. The flux of water through said material is preferably substantially unchanged as compared to the flux of water through said porous substratum. In another embodiment the substratum is non-porous and/or substantially non-penetrable to water.

There is also provided a material for use in a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation ex vivo, or a method of separating and/or isolating biological material ex vivo, or a method of producing a biohybrid organ ex vivo.

In another embodiment there is provided a for use in a diagnostic method carried out on the human or animal body, or for use in a method of therapy carried out on the human or animal body, or for use in a method of surgery carried out on the human or animal body.

There is also provided a material for use in a method of producing a biohybrid organ in vivo, and a material for use as a carrier for in vivo delivery of a medicament to a human or animal body in need of said medicament. In another embodiment there is provided a material for use in a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation in vivo, and a material for use in a method of separating and/or isolating biological material in vivo.

In another aspect there is provided a composition comprising the material according to the invention and a physiologically acceptable carrier. The invention also pertains to a pharmaceutical composition comprising the material according to the invention or the composition as defined herein and a pharmaceutically active ingredient and optionally a pharmaceutically active carrier.

There is also provided the use of the material or the composition or the pharmacetical composition according to the invention in a method of therapy carried out on the human or animal body, a method of surgery carried out on the human or animal body, or a diagnostic method carried out on the human or animal body.

In another embodiment there is provided the use of the material or the composition or the pharmacetical composition in a method of producing a biohybrid organ in vivo, or as a carrier for in vivo delivery of a medicament to a human or animal body in need of said medicament.

The invention also pertains to the use of the material or the composition or the pharmacetical composition in a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation in vivo, or use in a method of separating and/or isolating biological material in vivo, or use in a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation ex vivo, or use in a method of separating and/or isolating biological material ex vivo, or use in a method of producing a biohybrid organ ex vivoor use in the manufacture of an implantable organ or part thereof.

The material according to the invention may also be used as a carrier for a pharmaceutically active ingredient or a pharmaceutical composition.

There is also provided a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation ex vivo, said method comprising the steps of contacting a cell with the material or the composition or the pharmacetical composition according to the invention, and incubating said cell and said material under conditions allowing said cell to grow and/or proliferate and/or differentiate.

The invention also pertains to a method of separating and/or isolating biological material ex vivo, said method comprising the steps of contacting said biological material to be separated and/or isolated with the material or the composition or the pharmacetical composition according to the invention, and incubating said biological material and said material under conditions that allow separation and/or isolation.

There ia also provided a method of producing a biohybrid organ ex vivo, said method comprising the steps of contacting biohydrid organ cells with the material or the composition or the pharmacetical composition according to the invention, and incubating said biohybrid organ cells under conditions allowing the production of said biohybrid organ.

The invention also pertains to the following methods in particularly preferred embodiments:

Method of therapy carried out on the human or animal body, said method comprising the step of contacting said body with the material or the composition or the pharmacetical composition according to the invention.

Method of surgery carried out on the human or animal body, said method comprising the step of contacting said body the material or the composition or the pharmacetical composition according to the invention.

Method of diagnosis carried out on the human or animal body, said method comprising the steps of contacting said body with the material or the composition or the pharmacetical composition according to the invention, and detecting a signal generated directly or indirectly by said material.

Method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation in vivo, said method comprising the steps of contacting a cell with the material or the composition or the pharmacetical composition according to the invention, and incubating said cell and said material under conditions allowing said cell to grow and/or proliferate and/or differentiate.

Method of separating and/or isolating biological material in vivo, said method comprising the steps of contacting said biological material to be separated and/or isolated with the material or the composition or the pharmacetical composition according to the invention, and incubating said biological material and said material under conditions that allow separation and/or isolation.

Method of producing a biohybrid organ in vivo, said method comprising the steps of contacting biohydrid organ cells with the material or the composition or the pharmacetical composition according to the invention, and incubating said

biohybrid organ cells under conditions allowing the production of said biohybrid organ.

Method of in vivo delivery of a medicament to a human or animal body in need of said medicament, said method comprising the steps of contacting said body with the pharmacetical composition according to the invention and incubating said body contacted by said pharmaceutical composition under conditions allowing delivery of said medicament.

In another aspect there is provided a method for producing the material according to the invention, said method comprising the steps of providing a substratum having a second contact angle, and contacting said substratum with a composition comprising a plurality of macromolecules. The method preferably pertains to the production of a material as described herein above. The substratum preferably comprises a hydrophobic polymer and said substratum may be pretreated prior to being contacted by said macromolecule. The pretreatment is effective in increasing the wettability of said substratum.

The macromolecule according to the method comprises a hydrophilic polymer, preferably a latently reactivatable polymer. The macromolecule preferably has a molecular weight (MW) of more than 400 Dalton (Da). The macromolecule comprises a conjugate comprising a linkable head group, a linker group, a polymer chain, and a functional end group. The head group is preferably a photo-reactivatable aryl azide head group.

The macromolecule may optionally comprise a modifying agent, preferably a modifying agent capable of contacting said substratum and forming a self assembled monolayer.

According to the method for producing the material according to the invention, said method may comprising the further step of contacting said material with a first determinant comprising a biologically active compound. The biologically active

compound is preferably a polypeptide, an antibody, a polyclonal antibody, a monoclonal antobody, an immunogenic determinant, an antigenic determinant, a receptor, a receptor binding protein, an interleukine, a cytokine, a cellular differentiation factor, a cellular growth factor, or an antagonist to a receptor. The biologically active compound may be membrane associated and/or an extracellular matrix polypeptide natively produced by a microbial cell, a plant cell or a mammalian cell.

According to the method of the invention, a further step of contacting said material with a second determinant comprising a biological entity may also be included. The biological entity comprises a cell or a virus, or a part thereof, and said cell, or part thereof, is preferably selected from the group consisting of a mammalian cell, including a human cell and an animal cell, a plant cell, a microbial cell, including a eukaryotic microbial cell, including a yeast and a fungus, and a prokaryotic microbial cell including a bacteria. When being a virus, or part thereof, said virus is preferably selected from a mammalian virus, including a human virus and an animal virus, a plant virus, a microbial virus, including a eukaryotic microbial virus, including a yeast virus and a fungal virus, and a prokaryotic microbial virus including a bacteriophage. Accordingly, the biological entity as defined herein preferably comprises a polypeptide, or a part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof. The biological entity may also comprise an antibody, a polyclonal antibody, a monoclonal antobody, an immunogenic determinant, an antigenic determinant, a receptor, a receptor binding protein, an interleukine, a cytokine, a differentiation factor, a growth factor, or an antagonist to the receptor.

The method of producing a material according to the invention relates in one ~~preferred embodiment to a modification of a method described in U.S. Patent No.~~ 5,741,551 (to Guire). Accordingly, the novel biomaterial surface layer is in one preferred embodiment generated by a two-step process using e.g. macromolecular amphiphiles with latent (photo) reactivity. Consequently, in a first step, amphiphilic macromolecules are allowed to adsorb to a suitable polymer substratum. The

latent-reactive head-group will bring the amphiphils into reactive contact with the surface of the substratum. The hydrophilic main-body of the amphiphilic macromolecules exhibits a pronounced excluded volume leading to a lateral pattern of uniformly "self-assembled", adsorbed amphiphilic macromolecules. As described above, layer density and pattern depend on e.g. the amphiphilic character of the macromolecule such as e.g. chain length and/or degree of branching, the polymer substratum, as well as the solution conditions (e.g. concentration, solvent, salt, temperature). As a consequence, the interface properties will be adjustable by altering the molecular characteristics of both the polymer substratum and the macromolecule. Similar or at least substantially similar monolayer structures are attainable on even quite different substrata by adjusting e.g. macromolecular properties or solution conditions. Amphiphil adsorption can readily be monitored by known surface physico-chemical methods such as e.g. ellipsometry or contact angle (CA) measurements.

In a second step, excess of macromolecules is removed and the latently reactive head-groups are activated. The activation results in the formation of a covalent bond formation between the macromolecule and the surface of the polymer substratum. Activation is preferably achieved by using electromagnetic radiation in the ultraviolet (UV) or visible (Vis) light range.

In one preferred embodiment, the method according to the present invention is practiced on a substratum that has not been pretreated. Substrata such as solid surfaces may be pre-washed to remove surface contamination and may be modified as desired to affect solvophilic characteristics without adding functional groups that are involved in covalent bond formation with e.g. latent-reactive groups. For example, polystyrene surfaces may be washed and then exposed to hydroxyl ions in known water vapor plasma contact procedures so as to add hydroxyl groups to the substratum surface solely for the purpose of rendering the surface more readily wetted by aqueous solutions, the hydroxyl groups not being involved in subsequent covalent bond formation with the surface upon latent reactive group activation. Avoidance of pretreatment steps, defined in the definitions, leads not

only to important processing economies but also avoids technical problems associated with the attachment of bond-forming reactive groups to surfaces at uniform loading densities.

Examples

The following examples are illustrative of the present invention and will explain the invention in a non-limiting way.

Example 1

Synthesis of α-4-azidobenzoyl ω-methoxy (polyethylene glycol)s (ABMPEG).

4-Azidobenzoic acid is prepared from 4-aminobenzoic-acid which is diazotized with sodium nitrate.[26,27] The carboxylic acid is converted into the 4-azido benzoyl chloride with thionyl chloride.[26,27] 0,23 g (1,875 mmol) of DMAP in 10 ml dry methylene chloride is mixed with 0,17 ml (1,250 mmol) TEA. The solution is transferred into a 250 ml three neck roundbottom flask. After cooling down to 0°C, 0,57 g (3,125 mmol) 4-azido benzoyl chloride in 10 ml CH_2Cl_2 is added forming a yellow dispersion. 6,25 g (1,5 mmol) MPEG 5 kDa in 50 ml dry CH_2Cl_2 is added dropwise during 1 hour under dry nitrogen, after which the temperature is allowed to rise to room temperature. The reaction is continued with stirring overnight. The solution is filtered, and ABMPEG is precipitated in cold diethylether. The product is purified by two further precipitations from CH_2Cl_2/diethylether and dried in vacuum. Yield: 4,83 g (74 %).

The here described method is also applied for the synthesis of ABMPEG 2 kDa and 10.000 respectively using the same molar ratios.

Example 2

Adsorption characteristics/kinetics of ABMPEG 5 kDa and MPEG 5 kDa to a PSf surface monitored by ellipsometry

1. Preparation of polymer surfaces. Silica surfaces are prepared from polished silicon wafers which are thermally oxidized in pure and saturated oxygen followed by annealing and cooling under argon flow to yield an oxide layer of about 30 nm. Wafers are cut into rectangular slides (10-14 mm x 20-30 mm), thoroughly cleaned with detergent, etched for 15 min in a freshly mixed 3:1 (v:v) sulfuric acid (96 %): hydrogen peroxide (30 %) solution, thoroughly rinsed, stabilized for 2 hours and rinsed again with/in ultrapure H_2O. Slides are dried free of dust for two hours at 120°C. This procedure results in surfaces dense in silanol groups with a contact angle of less than 10°. In order to yield hydrophobic surfaces, cleaned slides are silanised in air saturated with hexamethyldisilazane (HMDS) at approx. 110°C. Excess HMDS is rinsed away with ultrapure H_2O. Slides are dried free of dust at room temperature. The silanised slides are spin-coated with a 3 % (w:w) polysulfone (PSf) in 1,2-dichlorbenzene solution. Slides are completely wetted by the polymer solution and then spun for 10 sec at 500 rpm and consecutively for 50 sec at 5.000 rpm in order to attain a smooth polymer film. Coated slides are dried for at least 4 hours at vacuum at 60°C.

2. Ellipsometry measurements. ABMPEG 5 kDa and MPEG 5 kDa adsorption out of aqueous solution to PSf spin-coated HMDS-treated silicon slides is monitored *in situ* using an automated Rudolph Thin Film ellipsometer, type 43603-200E, equipped with a thermostated quartz cuvette.[28] Spin-coated slides are stabilized in 4,5 ml water for at least 15 min or until constant polarizer and analyzer signals are obtained. 0,5 ml of concentrated aqueous ABMPEG/MPEG solution is added yielding 5 ml solution at defined concentration. A magnetic stirrer is activated for 30 sec upon addition of the ABMPEG/MPEG concentrate in order to homogenize the solution. Polarizer and analyzer data is collected until apparent equilibrium is reached. From the attained data, it is possible to calculate thickness and refractive

index of an adsorbed layer and/or its mass.[29] Adsorption data is calculated for approximated values of the partial specific volume and the ratio between the molar weight and the molar refractivity for both ABMPEG and MPEG respectively applying the same values for both species. Results for the calculated adsorbed mass are represented in arbitrary units as only approximated values of the partial specific volume and molar refractivity of ABMPEG and MPEG were at hand.

3. Results Fig.10 depicts adsorption kinetics monitored by ellipsometry for ABMPEG and MPEG respectively. Enhanced adsorption (factor = 3,5) and prolonged equilibrium times (> 2h) are observed for ABMPEG when compared with MPEG. The pronounced difference in the adsorptive characteristics of the two materials indicates a strong affinity between the aromatic head-group and the polymer surface. Flushing with water (20 ml/min) does not effect the adsorbed amount, i.e. no desorption of ABMPEG or MPEG takes place. This is an often observed characteristic of macromolecular adsorption related to multiple adsorption sites existent between macromolecule and surface.

Example SEQ3

Controlling polymer surface hydrophilicity and heterogeneity through photografting of ABMPEG

1. Preparation of polymer surfaces. Glass coverslips are cleaned with detergent, rinsed with ultrapure water, and etched for 15 min at approx. 40±5 °C in a freshly mixed 3:1 (v:v) sulfuric acid (96 %): hydrogen peroxide (30 %) solution. Coverslips are thoroughly rinsed, stabilized for 2 hours and rinsed again with/in ultrapure H_2O. Slips are dried free of dust for two hours at 120°C. n-octadecyldimethylchlorosilane (ODDMS) is grafted to the cleaned coverslips by immersing them in a 2 % (w:w) ODDMS in n-hexane solution for 1 hour at room temperature. Coverslips are rinsed twice with n-hexane and three times with ethanol and air dried at room temperature. The ODDMS-treated coverslips are spin-coated with a 3 % (w:w) polysulfone (PSf) in 1,2-dichlorbenzene solution. Coverslips are completely wetted

by the polymer solution and then spun for 10 sec at 500 rpm and consecutively for 50 sec at 5.000 rpm in order to attain a smooth polymer film. Coated coverslips are dried for at least 4 hours at vacuum at 60°C.

2. ABMPEG grafting to polymer surface. ABMPEG grafting includes the following two consecutive steps as illustrated in Fig.11. In a first adsorption step aqueous ABMPEG solution of different concentrations is placed on the PSf coated coverslip, covered and kept in the dark for at least 12 h but maximal 18 h. Thereafter coverslips are gently rinsed in ultrapure water, covered by water and immediately exposed to UV light for 1 min. For UV irradiation a 50 W high pressure mercury lamp (ORIEL) equipped with a condenser is used. The UV rich light passes a high-pass glass filter with a cut off at 320 nm yielding an intensity of 30 mW/cm^2. Certain indicated control surfaces are not exposed to UV irradiation. To remove non-covalently bond ABMPEG certain indicated sample surfaces were exposed over night to a 1:1 (v:v) water:isopropanol mixture (H_2O/IP), thoroughly rinsed with the same mixture and with ultrapure water thereafter.

3. Contact angle (CA) measurements. CAs on modified and unmodified PSf coated coverslips are measured using the captive bubble method, where an air bubble is injected from a syringe with a stainless steel needle onto the inverted sample surfaces under water. The diameter of the contact area between the PSf film and the bubbles is always greater than 3 mm. While the needle remains inside the bubble, advancing and receding angle measurements are realized with a goniometer fitted with a tilting stage by stepwise withdrawing/adding air from/to the captured bubble. At least ten measurements of different bubbles on at least three different locations are averaged to yield one measurement.

4. Results. Fig.12 shows advancing and receding CAs of PSf spin-coats modified with different concentrations of ABMPEG 10 kDa. Surfaces were exposed to UV irradiation but not rinsed with (H_2O/IP). Note that under the valid assumption that adsorbed ABMPEG layers are in the relevant time scales stable in aqueous environment (see results in Example 2) ABMPEG adsorption is monitored and not

its chemical grafting. With rising ABMPEG bulk concentrations decreasing advancing and receding CAs are observed while CA-hysteresis increases in the applied concentration range. The results indicate that ABMPEG adsorption is highly controllable and reproducible. Desired degrees of hydrophilicity and thus surface density of ABMPEG are attained by adjusting bulk ABMPEG concentrations during adsorption.

Fig.13 shows CAs of surfaces which were modified applying ABMPEGs of three different chain lengths. Again, surfaces were exposed to UV irradiation but not rinsed with H2O/IP thereafter. The same trend regarding degree and controllability of the attained hydrophilization of the underlying PSf is observed for all different chain lengths, but differences in CA-hysteresis are observed. CA-hysteresis values are in general lower for shorter chain lengths, and a clear maximum is seen especially for the lowest molecular weight ABMPEG in the applied concentration range. Thus longer chain lengths seem to induce more chemical and/or morphological heterogeneity manifested in increased CA-hysteresis.

Fig.14 shows receding CAs and CA-hysteresis of PSf surfaces which were modified with different mixtures of ABMPEG of two different chain lengths (ABMPEG 2 kDa and ABMPEG 10 kDa). Again, surfaces were exposed to UV irradiation but not rinsed with H2O/IP thereafter. Surfaces show a gradual change in surface properties. This result implies that mixtures of different ABMPEGs and/or ABMPEG derivatives can be applied in order to attain/design intermediate surface characteristics.

Fig.15 shows receding CAs of PSf surfaces modified with different concentrations of ABMPEG 10 kDa. Samples were exposed to UV irradiation and there CAs measured before and after over night rinsing with H2O/IP. The data characterizes the efficiency of the photo-grafting process in dependence of applied ABMPEG concentration. For ABMPEG concentrations higher than 10 g/l the effectiveness of the photoreactive grafting diminishes rapidly manifested in the reversibility of the hydrophilization upon rinsing with H2O/IP. This indicates a decrease in head-group

orientation towards the surface lowering chances for successful grafting. Increased solute-solute interactions at rising surface coverage might be responsible.

Example 4

Assay of protein adsorption to modified PSf membranes

1. Membrane modification. Rinsed pieces of circular cut PSf ultrafiltration membrane (type GR61PP, DOW) (132,6 cm^2) were stabilized and cleaned from packaging liquids by permeating at least 6 liter of ultrapure water at 0.4 MPa for at least 1 h. The membrane was then cut into circular membranes of 25 mm diameter and their skin-layer modified with ABMPEG 5 kDa as described in Example 3. After exposure to UV irradiation were membranes exposed to ultrasound for 5 min and thoroughly rinsed thereafter.

2. Protein adsorption. The skin-layer of unmodified or modified membranes is contacted for 2 hours with a 1 g/l BSA solution (0.15 molar phosphate buffer, pH=7, room temperature), flushed with buffer and dried at 60°C over night. Adsorbed amount of protein (BSA) is determined by its total hydrolysis and consecutive amino acid analysis.[30)]

3. Results. Fig.16 shows adsorbed amounts of BSA in dependence of the applied ABMPEG concentration. BSA adsorption decreases for increasing ABMPEG concentration. Maximum reduction in comparison to an unmodified reference membrane of about 70 % is attained for the highest applied ABMPEG concentration of 10 g/l.

Example 5

Fibronectin Adsorption Measured by *In Situ* Ellipsometrie.

1.Procedure. FN (human plasma, lyophilized, MW 440 kD; Boehringer Mannheim, Germany) is reconstituted in phosphate-buffered saline (PBS; 5.8 mM Na_2HPO_4/NaH_2PO_4, 150 mM NaCl, pH = 7.4) containing 0.02 % (w/v) sodium azide

giving a concentration of about 0.12 g/l. The instrumental setup and the measurement procedure are identical to the one described in Example 2.1 and 2.2 respectively. PSf films on silicon wafers, modified with ABMPEG 10 kDa at different concentrations as described under Example 3.1 are placed in the quartz cuvette and stabilized in 2.5 ml PBS buffer for at least 15 min or until constant polarizer and analyzer signals are obtained. 0.5 ml of the concentrated FN solution is added yielding 3 ml with a defined concentration of 0.02 g/l. The magnetic stirrer is activated for 2-3 sec upon addition of the protein concentrate in order to homogenize the solution. After 30 min the cuvette is flushed for 10 min with PBS buffer using preinstalled tubings and a flow rate of 20 ml/min.

In the calculations of the amount of protein adsorbed, the different layers, silicon support, silicon oxide, ODMS-layer, PSf-film, and tethered ABMPEG are treated as one optical unit with an effective refractive index. The molar refractivity of FN is calculated as the sum of the individual molar refractivities of all amino acids in FN using tabulated values from{1100} yielding a value of 3.99 g/ml. For the partial specific volume of FN the value 0.75 ml/g is used.

Even if plateau values are typically observed after 1-2 hours (or much longer), it is possible to describe qualitatively protein – substratum interactions.

Fig.17 shows that all data curves follow the expected monotonic rise; FN desorption upon flushing is not observed. The adsorbed amount of FN decreases with higher degrees of ABMPEG surface functionalization and thus correlates qualitatively with the CA decrease shown in Fig.12 Maximum adsorption of almost 1.2 g/cm^2 is attained for both, unmodified PSf and PSf modified with the lowest ABMPEG concentration, i.e. 0.001 g/l. The adsorbed FN amount decreases by more than 60 % to 0.45 g/cm^2 for an ABMPEG concentration of 10 g/l. As shown in Example 4, yielded albumin adsorption to PSf UF membrane surfaces photo-grafted with ABMPEG 5 kDa and quantified by total hydrolysis and consecutive amino acid analysis of the adsorbed protein very similar results: The relative reduction depending on the degree of functionalization correlates very well with the here presented results for FN.

Example 6

Fibroblast adhesion to PSf and ABMPEG modified PSf surfaces: number of adherent cells, overall cell morphology and focal adhesion formation

1. Cells. Human fibroblasts were obtained from fresh skin biopsy and used up to the 9th passage. The cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10 % fetal bovine serum (FBS, Sigma Chemicals Co., St. Louis, MO) in an humidified incubator with 5 % CO_2. Fibroblasts from nearly confluent cultures were harvested with 0.05 % trypsin/0.6 mM EDTA (Sigma), and trypsin was neutralized with FBS.

2. Number of adherent cells and their morphology. Adhesion of fibroblasts was carried out in 6-well tissue culture plates containing the unmodified and modified PSf coated glass slides. Experiments were performed without or with pre-coating of the surfaces with fetal bovine serum (FBS, Sigma) for 30 min at 37°C. Approximately 105 cells in DMEM were pipetted into each well and incubated for 2 h at 37 C in a humidified CO_2 incubator. The number of adherent cells and their morphology was studied and photographed directly from the wells with an inverted phase contrast microscope Telaval 31 (Carl Zeiss, Germany). The mean number of adherent cells was determined by evaluating approx. 30 different randomly chosen microscopic fields on each surface. Cell counts were normalized to: *number of cells* per mm^2; the standard deviation was determined for each set of fields on a surface.

3. Focal adhesions formation. Focal adhesions were visualized by immunofluorescence. Samples were processed as follows: Attached cells were fixed with paraformaldehyde (3 %) for 10 min and permeabilized with 0,2 % Triton X-100 for 5 min. To detect focal adhesions, samples were incubated for 30 min at 37°C with monoclonal anti vinculin antibody (Sigma Immunochemicals, St- Louis, MI, USA), followed by Cy3 conjugated goat anti mouse secondary antibody (Jackson Immuno Research, Inc. West Growe, PA, USA). Samples were mounted

with Mowiol, and viewed and photographed with a inverted fluorescent microscope Axiovert 100 (Carl Zeiss, Germany).

4. Results. Fig.18 shows the number of adherent cells, and a clear maximum is seen for PSF surfaces modified with ABMPEG solutions of intermediate concentrations.

Fig.19 demonstrates significantly improved cell morphology and spreading on PSF surfaces modified with intermediate concentration of ABMPEG (0.001 g/l and 0.01 g/l), in comparison to unmodified PSf, or PSF modified with comparable high concentrations of ABMPEG (1 g/l and 10 g/l). Fig.20 represents the optimal focal adhesions formation on surfaces modified with intermediate concentration of ABMPEG (0.001 g/l (b), and 0.01 g/l (c)). At 0.1 g/l (d), focal adhesions already start to disorganize and almost completely disappear at 10 g/l (f). An important observation is that the effect of PEG density on focal adhesions formation is much more pronounced on serum coated PEG surfaces (see Fig.21, and compare with Fig.20)

Example 7

Fibronectin (FN) matrix of human fibroblasts adhering on different ABMPEG surfaces

1. FN matrix formation. Approximately 5 x 10^5 cells in 3 ml medium containing 10 % FBS were incubated for 5 days in 6-well tissue culture plates (Falcon, Becton Dickenson & Company, New Jersey) containing the PSf coated and photo-modified glass slides. At the end of the incubation cells were fixed with 3 % paraformaldehyde and FN matrix deposited on the different surfaces was visualized by immunofluorescence using a specific anti human FN matrix mouse monoclonal antibody (Immunotech SA, France, lot No 0326), followed by Cy3-conjugated goat anti mouse secondary IgG1 antibody (Jackson Immuno Research,

Inc. West Growe, PA, USA). Further investigations and photography was carried out with an inverted fluorescence microscope as above.

2. Results. Fig.22 demonstrates maximal FN matrix formation of fibroblasts cultured on surfaces with moderate PEG density. Note, the secreted FN was also highly organized on these surfaces (see Fig.23)

References

1) Andrade, J.D., Hlady, V., Protein adsorption and materials biocompatibility: A Tutorial Review and Suggested Hypotheses, *Adv.Polym.Sci.* **79**, 1 (1986).

2) Norde, W., Lyklema, J., Why proteins prefer interfaces, *J.Biomater.Sci.Polymer Edn.* **2**, 183 (1991).

3) Grinnell, F., Cellular adhesiveness and extracellular substrata, *Int. Rev. Cytol.* **53**, 65 (1978).

4) Grinnell, F., Fibronectin adsorption on material surfaces, in: *Blood in contact with natural and artificial surfaces,* 1st ed., E.F. Leonard Ann.N.Y.A.Sci. New York (1987), 280ff.

5) Grinnell, F., Feld, M., Fibronectin adsorption on hydrophilic and hydrophobic surfaces detected by antibody binding and analyzed during cell adhesion in serum containing medium, *J.Biol.Chem.* **257**, 4888 (1982).

6) Hynes, R.O., Integrins: A family of cell surface receptors, *Cell* **48**, 549 (1987).

7) Hynes, R.O., Integrins: Versatility, modulation and signaling, *Cell* **69**, 11 (1992).

8) Fabrizius-Homan, D.J., Cooper, S.L., A comparison of the adsorption of three adhesive proteins to biomaterial surfaces, *J.Biomater.Sci.Polymer Edn.* **3**, 27 (1991).

9) Juliano, D.J., Saavedra, S.S., Truskey, G.A., Effect of the conformation and orientation of adsorbed fibronectin on endothelial cell spreading and the strength of adhesion, *J.Biomed.Mat.Res.* **27**, 1103 (1993).

10) Groth, T., Zlatanov, I., Altankov, G., Adhesion of human peripheral lymphocytes on biomaterials preadsorbed with fibronectin and vitronectin, *J.Biomater.Sci.Polymer Edn.* **6**, 729 (1994).

11) Saltzman, W.M., Cell interactions with polymers, in: *Principles of tissue engineering,* 1st ed., R. Lanza R.G. Landes Company, (1997), 225ff.

12) Colton, C.K., Implantable biohybrid artificial organs, *Cell Transplantation* **4**, 415 (1995).

13) Lee, J.H., Lee, J.W., Khang, G., Lee, H.B., Interactions of cells on chargeable functional group gradient surfaces, *Biomaterials* **18**, 351 (1997).

14) Massia, S.P., Hubbell, J.A., Covalent surface immobilization of Arg-Gly-Asp- and Tyre-Ile-Gly-Ser-Arg-containing peptides to well defined cell adhesive substrates, *Analy.Biochem.* **187**, 155 (1990).

15) Braber den, E.T., Ruijter de, J.E., Smits, H.T.J., Ginsel, L.A., Recum, A.F., Jansen, J.A., Effect of parallel surface microgrooves and surface energy on cell growth, *J Biomed Mater Res* **29**, 511 (1995).

16) Curtis, A., Wilkinson, C., Topographical control of cells, *Biomaterials* **18**, 1573 (1997).

17) Chan, C.M., Contact angle measurements, in: *Polymer surface modification and characterization*, 1st ed.,Hanser Publishers, München, (1994), Chapter 2, 193ff

18) USA, 5128170, (1992), Matsuda, T., Inoue, K., Kani, N..

19) USA, 5476509, (1995), Keogh, J.R..

20) USA, 5330911, (1994), Hubbell, J.A., Massia, S.P., Desai, N.P..

21) USA, 5593814, (1997), Matsuda, T., Inoue, K., Tani, N..

22) USA, 5512474, (1996), Clapper, D.L., Hu, W.S..

23) Nilsson, J.L., Protein fouling of UF membranes: Causes and Consequences, *J.Membrane Sci.* **52**, 121 (1990).

24) Ulbricht, M., Matuschewski, H., Oechel, A., Hicke, H.G., Photo-induced graft polymerization surface modifications for the preparation of hydrophilic and low-protein-adsorbing ultrafiltration membranes, *J.Membrane Sci.* **115**, 31 (1996).

25) Holmberg, K., Tiberg, F., Malmsten, M., Brink, C., Grafting with hydrophilic polymer chains to prepare protein-resistant surfaces, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **123**, 297 (1997).

26) Ulbricht, M., Hicke, H.G., Photomodifizierung von Ultrafiltrationsmembranen, I. Photochemische Modifizierung von Polyacrylnitril-Ultrafiltrationsmembranen mit Arylaziden, *Angew.Makromol.Chem.* **210**, 69 (1993).

27) Ulbricht, M., Hicke, H.G., Photomodifizierung von Ultrafiltrationsmembranen, II. Ultrafiltrationseigenschaften von mit Arylaziden modifizierten Polyacril-Ultrafiltrations Membranen, *Angew.Makromol.Chem.* **210**, 97 (1993).

28) Arnebrant, T., Ph.D.-thesis, Lund University, Lund, Sweden, (1987).

29) McCrackin, F.L., Passaglia, E., Stromberg, R.R., Steinberg, H.L., *J Res Nat Bur Stand Sect A* **67**, 363 (1963).

30) Barkholt, V., Jensen, A.L., Amino acid analysis: determination of cysteine plus half-cystine in proteins after hydrochloric acid hydrolysis with a disulfide ompound as additive, *Analy.Biochem.* **177**, 318 (1989).

31) Sofia, S.J., Premnath, V., Merrill, E.W. Poly(ethylene oxide) grafted to silicon surfaces: grafting density and protein adsorption, *Macromolecules* **31**, 5059 (1998).

32) Williams, D.F. *Definitions in biomaterials,* Elsevier, Amsterdam (1987).

33) Merrit, M.V., Mrksich, M., Whitesides, G.M. Using self-assembled monolayers to study the interactions of man-made materials with proteins, in: *Principles of tissue engineering,* 1st ed., R. Lanza R.G. Landes Company, (1997). 225ff.

34) Garbassi, F., Morra, M., Occhiello, E. Modification techniques, in: *Polymer surfaces - from physics to technology,*Anonymous. John Wiley & Sons Ltd, Chapter 6-8, Chichester (England) (1994). 221ff.

35) Ratner, B.D. Surface modification of polymers for **biomaedical** applications: Chemical, biological and surface analytical challenges, in: *Surface modification of polymeric biomaterials,* 1st ed., B.D. Ratner Plenum Press, New York (1997). 1ff.

36) Andrade, J.D., Hlady, V. Protein Adsorption and Materials Biocompatibility: A Tutorial Review and Suggested Hypotheses, *Adv.Polym.Sci.* **79**, 1 (1986).

37) Ulbricht, M., Papra, A. Polyacrylonitrile enzyme ultrafiltration membranes prepared by adsorption, crosslinking, and covalent binding, *Enzyme Microb.Technol.* **20**, 61 (1997).

38) Szleifer, I. Polymers and proteins: interactions at interfaces, *Current Opinion in Solid State & Materials Science* **2**, 337 (1997).

39) Sofia, S.J., Premnath, V., Merrill, E.W. Poly(ethylene oxide) grafted to silicon surfaces: grafting density and protein adsorption, *Macromolecules* **31**, 5059 (1998).

40) Schröen, C.G.P.H., Cohen Stuart, M.A., Voort Maarschalk van der, K., Padt van der, A., Riet van't, K. Influence of preadsorbed block copolymers on protein adsorption: surface properties, layer thickness, and surface coverage, *Langmuir* **11**, 3068 (1995).

41) Azzam, R.M.A., Bashara, N.M. *Ellipsometry and polarized light,* 3rd ed., Elsevier Science, Amstaerdam (1987).

42) Arnebrant, T. Lund University, Lund, Sweden, (1987).

43) Cuypers, P.A., Corsel, J.W., Janssen, M.P., Kop, J.M.M., Hermens, W.T., Hemker, H.C. The adsorption of prothrombin to phosphatidylserine multilayers quantitated by ellipsometry, *J Biol Chem* **258**, 2426 (1983).

Legends to Figures

Fig. 1 Illustration of the effect of adsorption of a biological material on a surface unable to support said material in its native conformation.

Fig. 2

Fig. 3 Illustration of a substratum comprising an adsorbed polypeptide stabilized in a biologically active form such as an active conformation by surface anchored macromolecules exhibiting an excluded volume.

Fig. 4 Illustration of contact angle measurement.

Fig. 5 Illustration of the attachment to a substratum of a macromolecule by means of a chemical bond, wherein said macromolecule is stabilized in a biologically active form such as an active conformation by surface anchored macromolecules exhibiting an excluded volume.

Fig. 6 Illustration of the attachment to a substratum of a macromolecule by means of a ionic bond, wherein said macromolecule is stabilized in a biologically active form such as an active conformation by surface anchored macromolecules exhibiting an excluded volume.

Fig. 7 Illustration of the attachment to a substratum of a macromolecule in the form of an adsorbed entity, wherein said macromolecule is stabilized in a biologically active form such as an active conformation by surface anchored macromolecules exhibiting an excluded volume.

Fig. 8 Illustration of the attachment to a substratum of a macromolecule in the form of an entangled entity, wherein said macromolecule is stabilized in a biologically active form such as an active conformation by surface anchored macromolecules exhibiting an excluded volume.

Fig. 9 Illustration of the attachment to a self-assembled monolayer (SAM) of a macromolecule, wherein said macromolecule is stabilized in a biologically active form such as an active conformation by surface anchored macromolecules exhibiting an excluded volume.

Fig.10 Adsorption kinetics of ABMPEG 5 kDa and MPEG 5 kDa out of aqueous solutions (10 g/l) onto PSf spin-coats on polished silica wafers determined by ellipsometry.

Fig.11 Polymer surface functionalization procedure.

Fig.12 Advancing and receding CAs of modified PSf spin-coats on glass cover slips being modified with different solution concentrations of ABMPEG 10 kDa bulk.

Fig.13 Receding CAs and their hysteresis on PSf spin-coats on glass cover slips modified with different solution concentrations of ABMPEG 10 kDa, ABMPEG 5 kDa, and ABMPEG 2 kDa.

Fig.14 Advancing and receding CAs of PSf spin-coats on glass cover slips modified with mixtures of ABMPEG 2 kDa and ABMPEG 10 kDa yielding a total ABMPEG solution concentration of 10 g/l.

Fig.15 PSf spin-coats on glass coverslips are modified with different solution concentrations of ABMPEG 10 kDa. Receding CAs are shown after modification and after consecutive rinse with isopropanol/water = 1/1.

Fig.16 Adsorbed amount of BSA on a PSf UF membrane, modified with different solution concentrations of ABMPEG 5 kDa, after 2 h static exposure of the membrane to a 1 g/l BSA buffer-solution (0.15 molar phosphate buffer, pH = 7, room temperature) and consecutive gentle rinsing with buffer.

Fig.17 FN adsorption onto PSf spin-coated silicon wafers modified with different solution concentrations of ABMPEG 10 kDa as indicated at the data curves. Arrow indicates the start of the flushing with buffer.

Fig.18 Mean number of fibroblasts per mm^2 adhering on different PSf spin-coats on glass coverslips modified with different solution concentrations of ABMPEG 10 kDa. Effect of ABMPEG 10 kDa concentration. Fibroblasts were plated for 2 h onto the different surfaces. At the end of incubation, samples were evaluated microscopically under phase contrast. Error bars indicate standard deviations.

Fig.19 Overall cell morphology of fibroblasts adhering on different PSf spin-coats on glass coverslips modified with different solution concentrations of ABMPEG 10 kDa. Effect of ABMPEG 10 kDa concentration. Fibroblasts were plated for 2 h on unmodified PSf (a), or PSf grafted with ABMPEG 10 kDa at concentrations (in g/l) as follows: (b) 0.001, (c) 0.01, (d) 0.1, (e) 1, (f) 10. At the end of incubation, samples were investigated and photographed under phase contrast at low magnification 20X.

Fig.20 Focal adhesion formation of fibroblasts adhering on different PSf spin-coats on glass coverslips modified with different solution concentrations of ABMPEG 10 kDa. Effect of ABMPEG 10 kDa concentration. Cells were plated for 2 h on unmodified PSf (a), or PSF modified with ABMPEG 10 kDa with concentrations (in g/l) as follows: (b) 0.001, (c) 0.01, (d) 0.1, (e) 1, (f) 10. At the end of incubation, the cells were fixed, permeabilized and stained for vinculin by immunofluorescence. Samples were visualized and photographed at high magnification 100X.

Fig.21 Focal adhesion formation of fibroblasts adhering on different PSf spin-coats on glass coverslips modified with different solution

concentrations of ABMPEG 10 kDa. Effect of serum pre-coating. Cells were plated for 2 h on serum-coated unmodified PSf (a), or on serum-coated PSf prior modified with ABMPEG 10 kDa with concentrations (in g/l) as follows: (b) 0.001, (c) 0.01, (d) 0.1, (e) 1, (f) 10. At the end of incubation, the samples were fixed, permeabilized and stained for vinculin. Samples were visualized and photographed at high magnification.

Fig.22 FN matrix formation by human fibroblasts cultured on different PSf spin-coats on glass coverslips modified with different solution concentrations of ABMPEG 10 kDa. Effect of ABMPEG 10 kDa concentration. Cells were cultured for 5 days in DMEM containing 10% FBS on (a) unmodified PSf, or PSf modified with ABMPEG 10 kDa with concentrations (in g/l) as follows: (b) 0.001, (c) 0.01, (d) 0.1, (e) 1 and (f) 10. At the end of incubation, the cells were fixed and stained for FN by immunofluorescence. Samples were viewed and photographed at low magnification 25X (see Material and Methods).

Fig.23 FN matrix formation by human fibroblasts cultured on different PSf spin-coats on glass coverslips modified with different solution concentrations of ABMPEG 10 kDa. Effect of ABMPEG 10 kDa concentration. Cells were cultured for 5 days in DMEM containing 10% FBS on different PEG surfaces as follows: (a) unmodified PSf, or PSf modified with ABMPEG 10 kDa with concentrations (in g/l) as follows: (b) 0.001, (c) 0.0.1, and (d) 10. At the end of incubation, the cells were fixed and stained for FN by immunofluorescence. Samples were viewed and photographed at high magnification 100X (see Material and Methods).

Figures

Fig.1

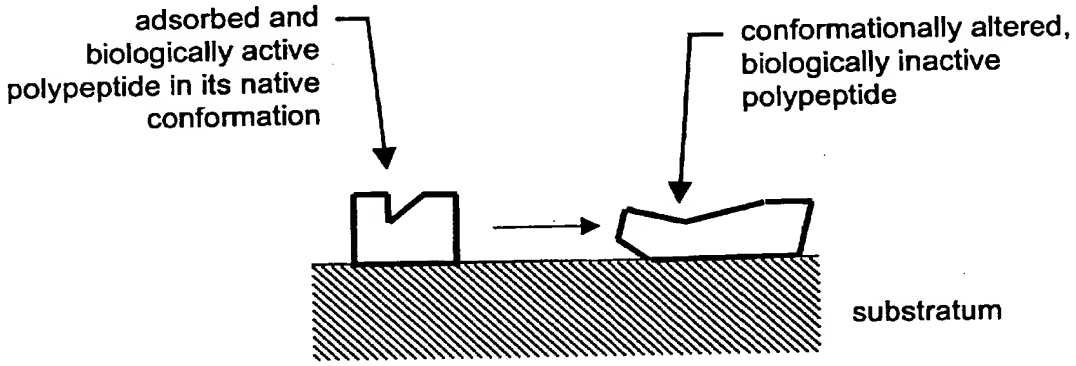


Fig.3

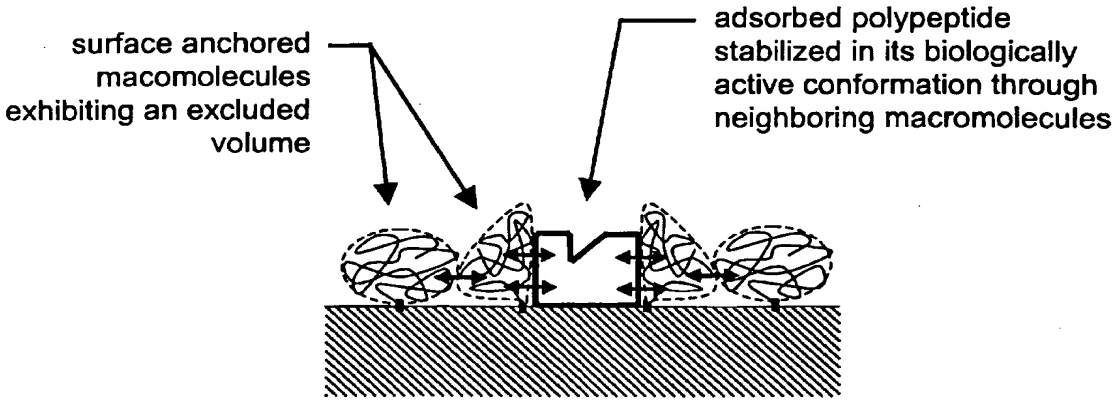

surface anchored macomolecules exhibiting an excluded volume
adsorbed polypeptide stabilized in its biologically active conformation through neighboring macromolecules
substratum

Fig.4

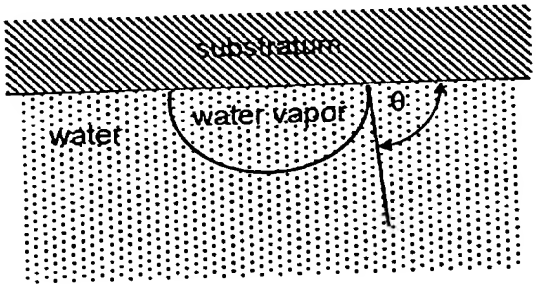


Fig.5

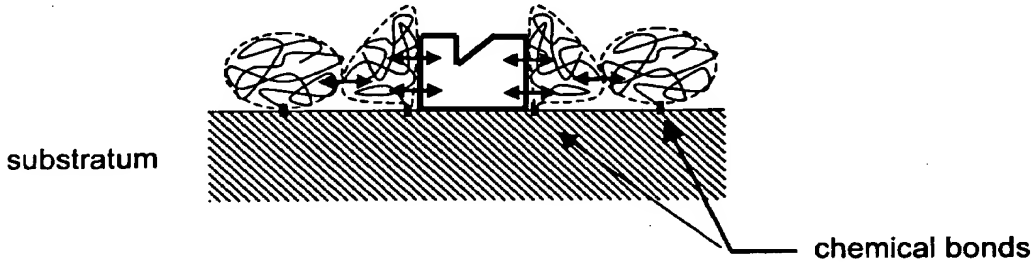


Fig.7

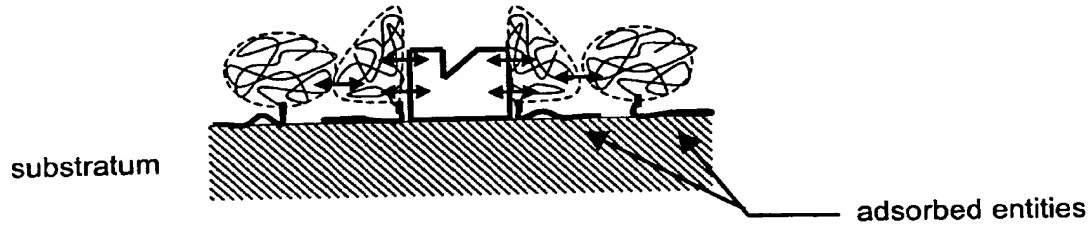


Fig.6

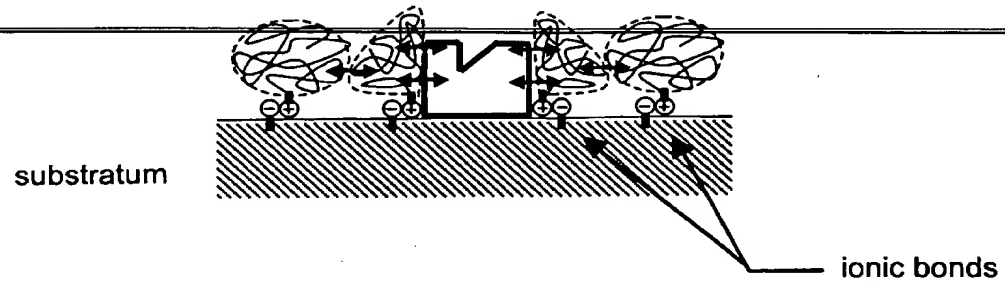


Fig.8

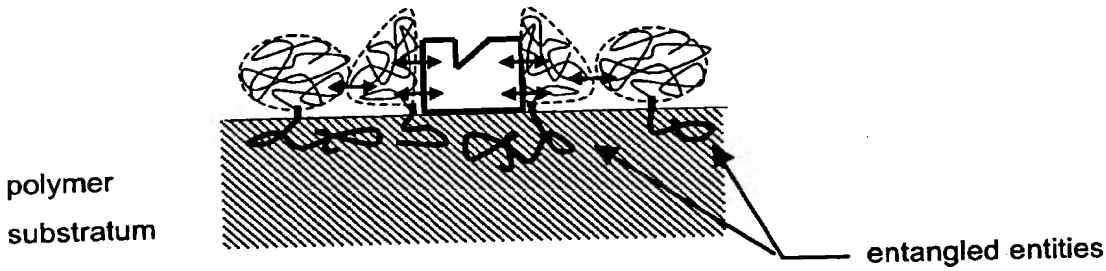


Fig.9

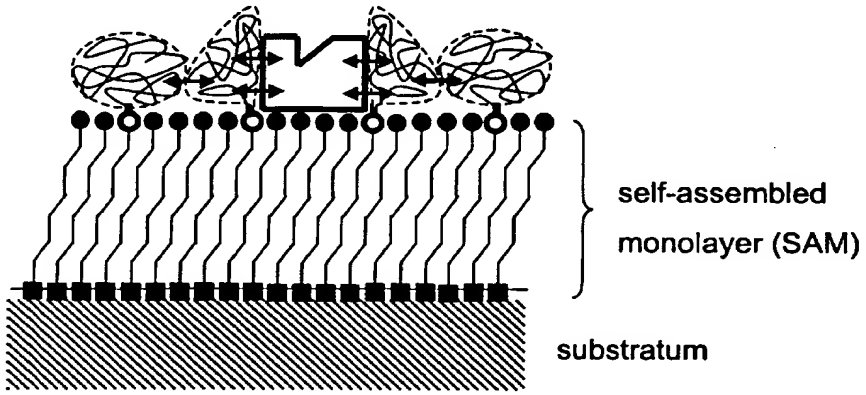


Fig.10

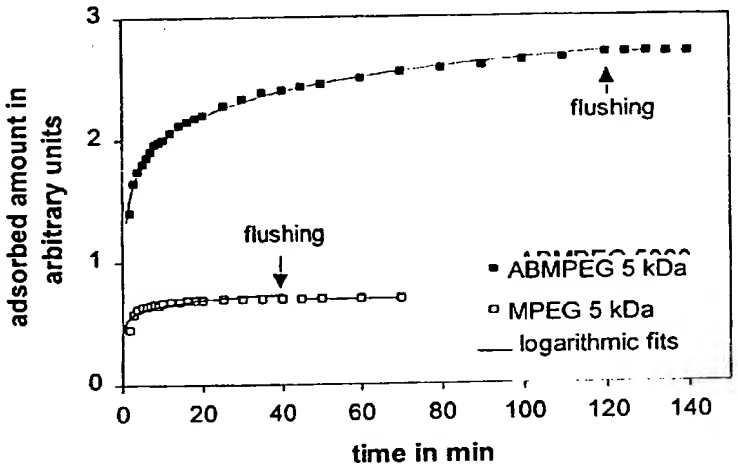
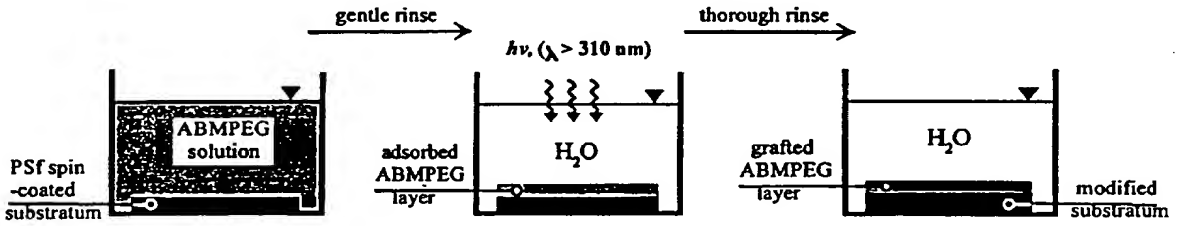

adsorbed amount in arbitrary units
time in min
flushing
flushing
ABMPEG 5 kDa
MPEG 5 kDa
logarithmic fits
0
1
2
3
0
20
40
60
80
100
120
140

Fig.11

Fig.11



1. ABMPEG adsorption 2. Exposure to UV-light

Fig.12

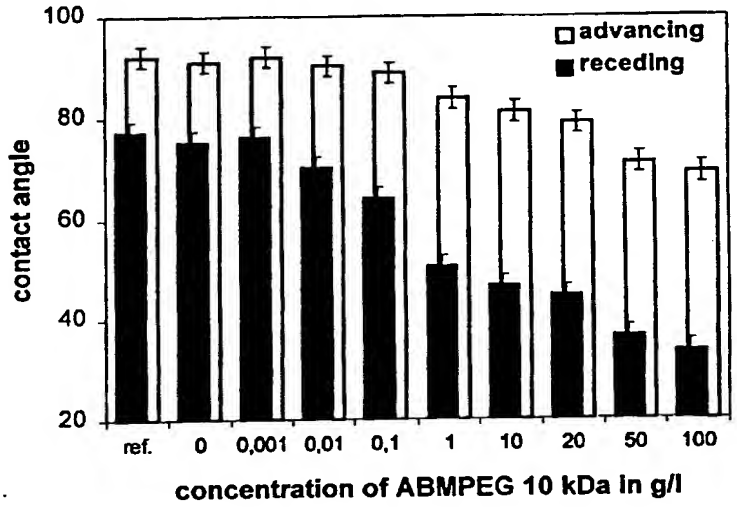

100
80
60
40
20
contact angle
advancing
receding
ref.
0
0,001
0,01
0,1
1
10
20
50
100
concentration of ABMPEG 10 kDa in g/l

Fig.13

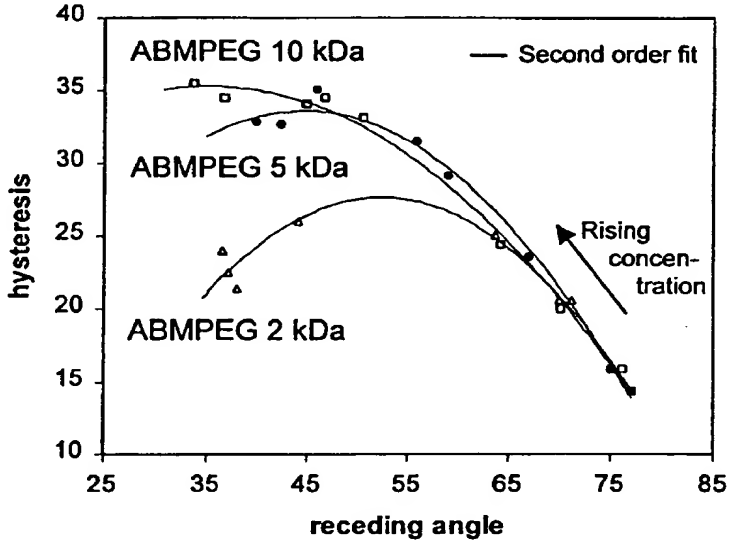

ABMPEG 10 kDa
Second order fit
ABMPEG 5 kDa
ABMPEG 2 kDa
Rising concen-tration
hysteresis
receding angle
40
35
30
25
20
15
10
25
35
45
55
65
75
85

Fig.14

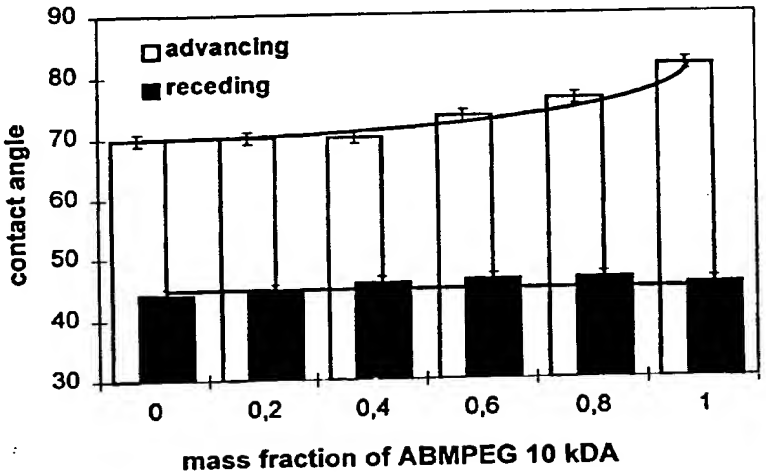

90
80
70
60
50
40
30
contact angle
advancing
receding
0
0,2
0,4
0,6
0,8
1
mass fraction of ABMPEG 10 kDA

Fig.15

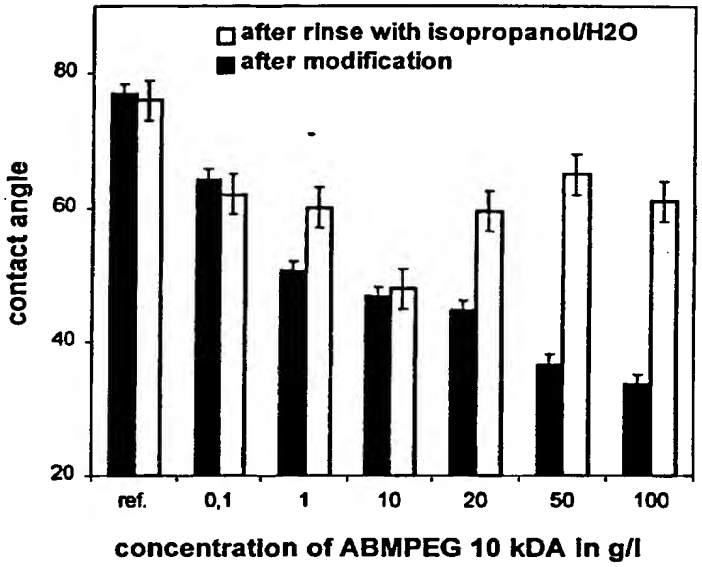

after rinse with isopropanol/H2O
after modification
contact angle
80
60
40
20
ref.
0,1
1
10
20
50
100
concentration of ABMPEG 10 kDA in g/l

Fig.16

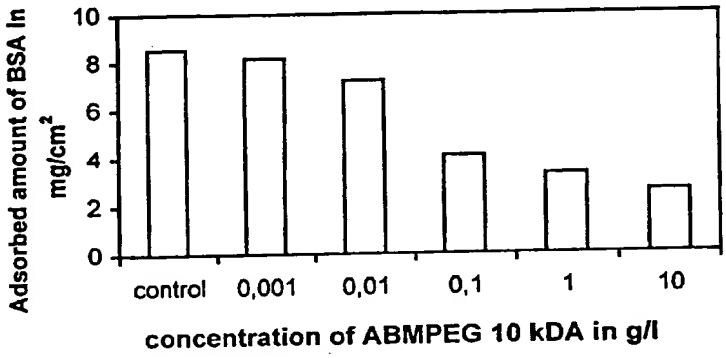

Adsorbed amount of BSA in mg/cm²
10
8
6
4
2
0
control
0,001
0,01
0,1
1
10
concentration of ABMPEG 10 kDA in g/l

Fig.17

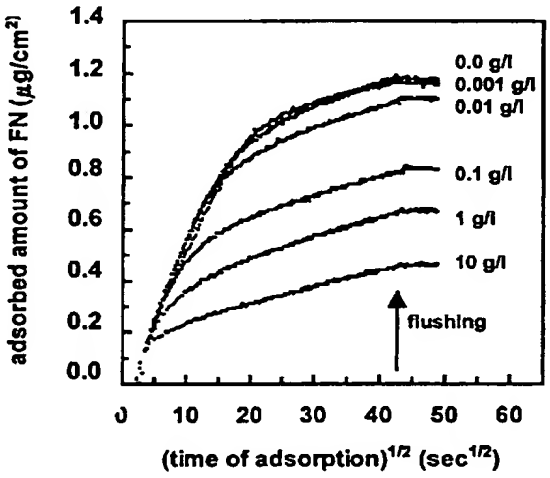

adsorbed amount of FN (µg/cm²)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0 g/l
0.001 g/l
0.01 g/l
0.1 g/l
1 g/l
10 g/l
flushing
0
10
20
30
40
50
60
(time of adsorption)^1/2 (sec^1/2)

Fig.18

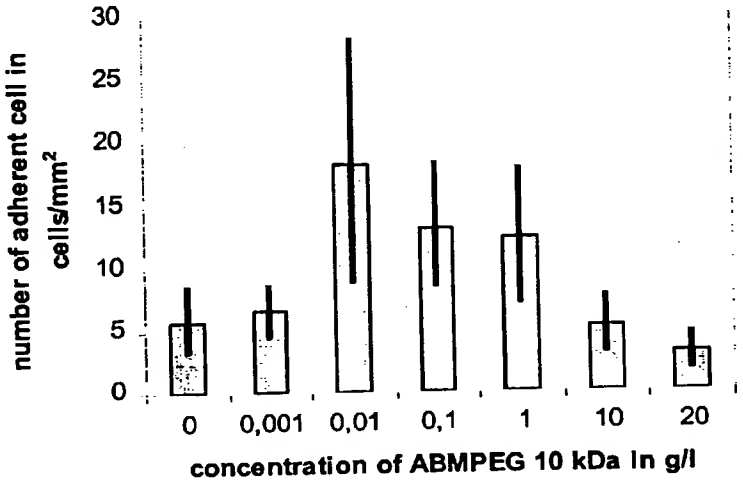

30
25
20
15
10
5
0
number of adherent cell in cells/mm²
0
0,001
0,01
0,1
1
10
20
concentration of ABMPEG 10 kDa in g/l

Fig. 19

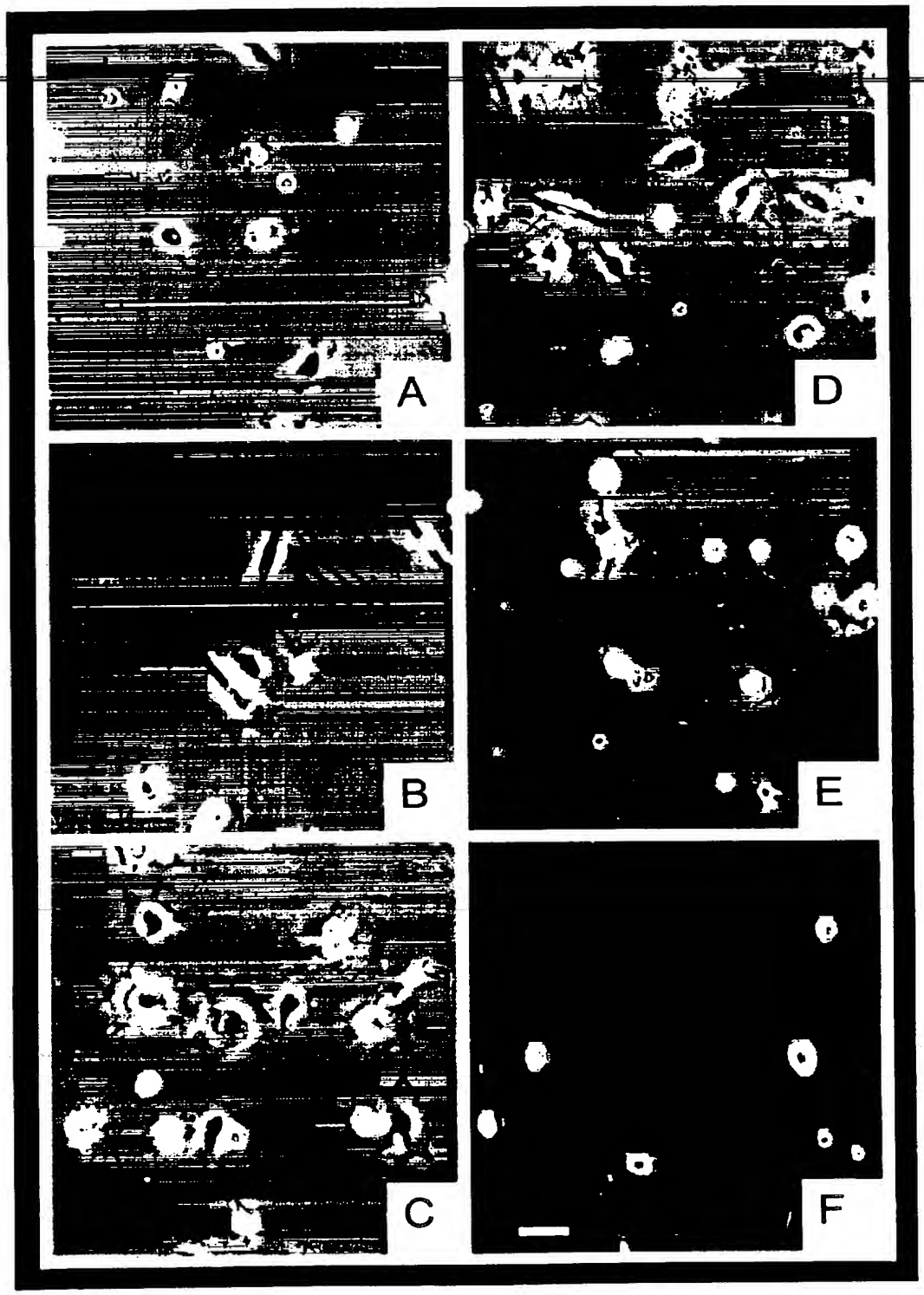


Fig. 20

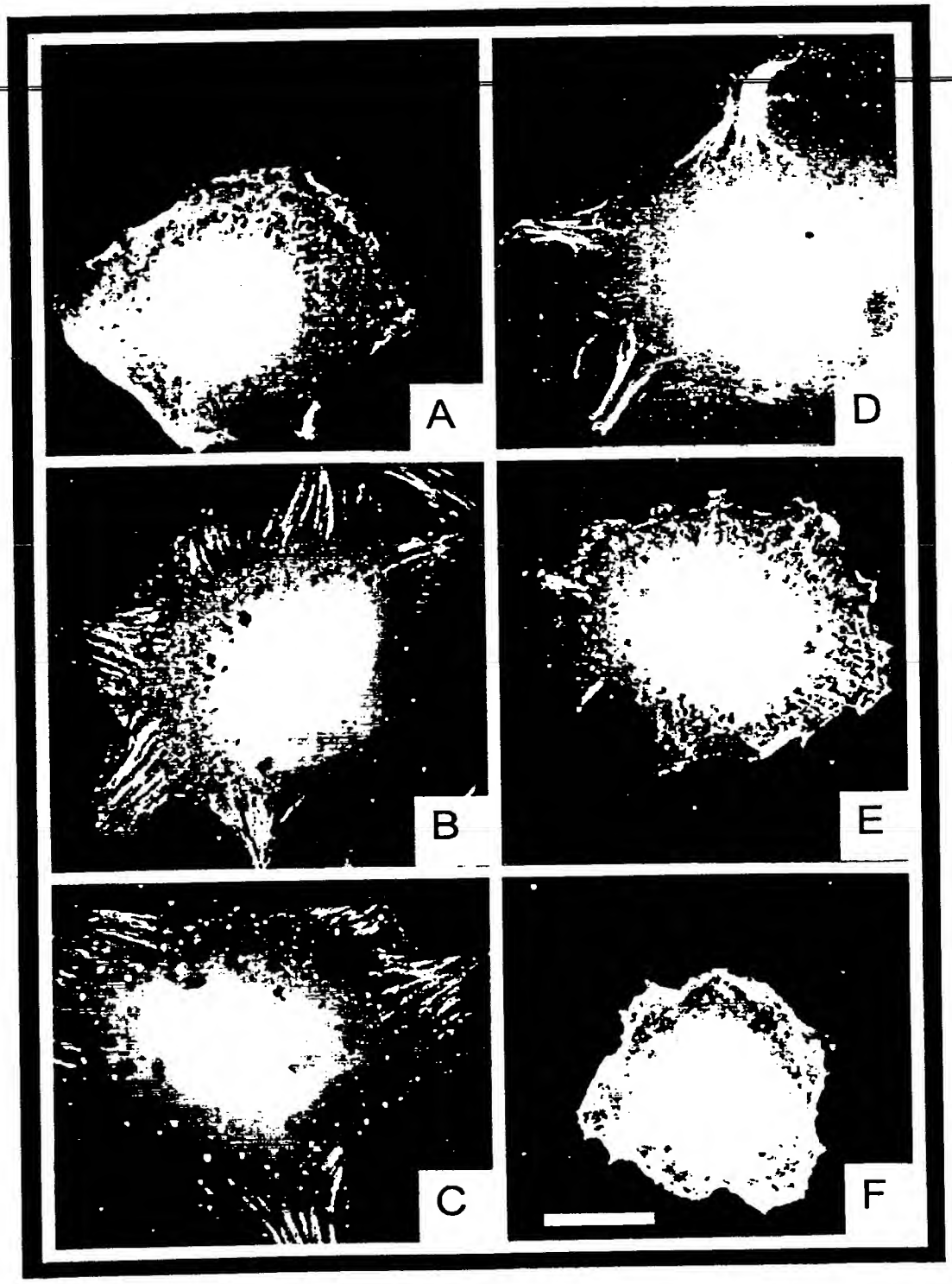


Fig. 21

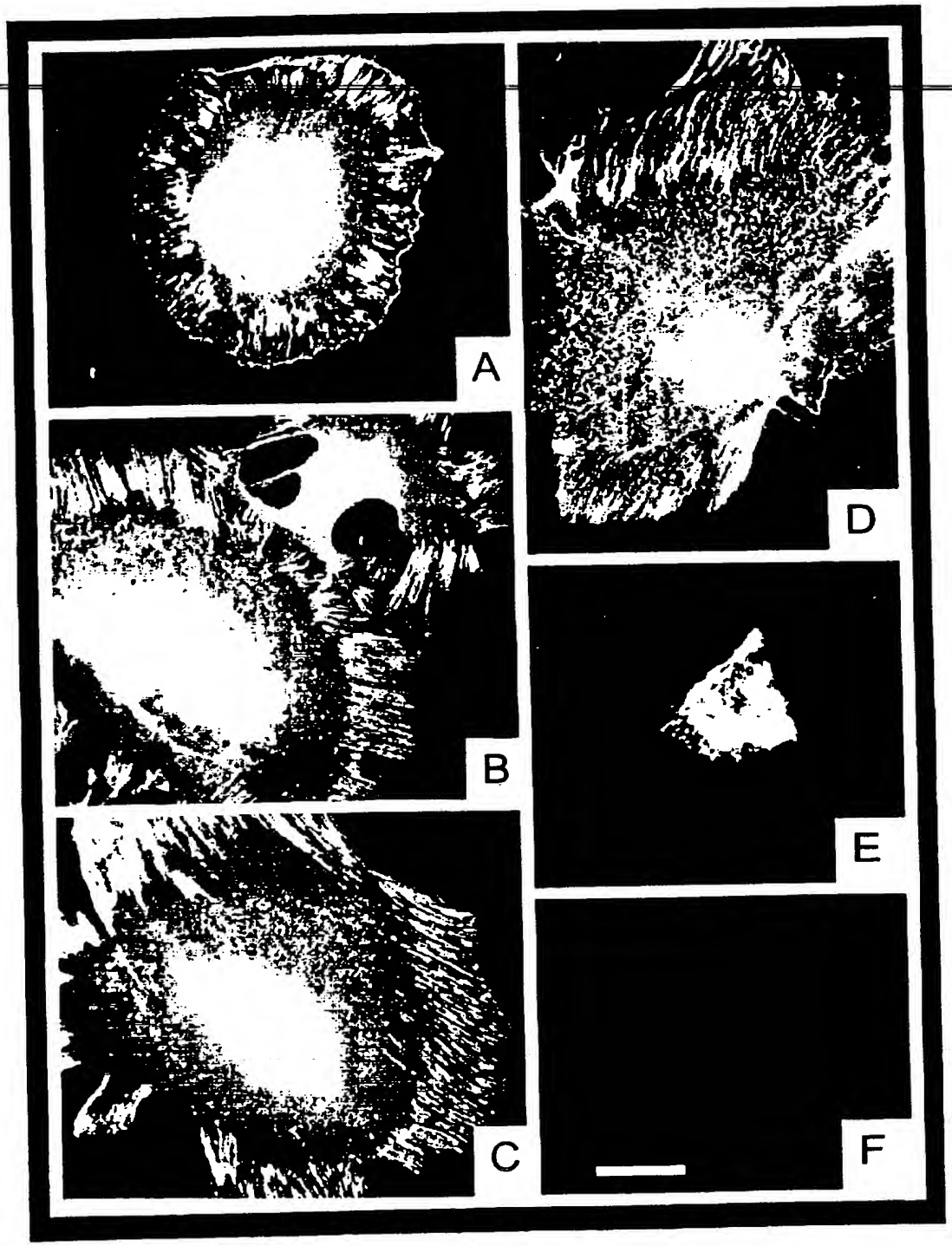


Fig. 22

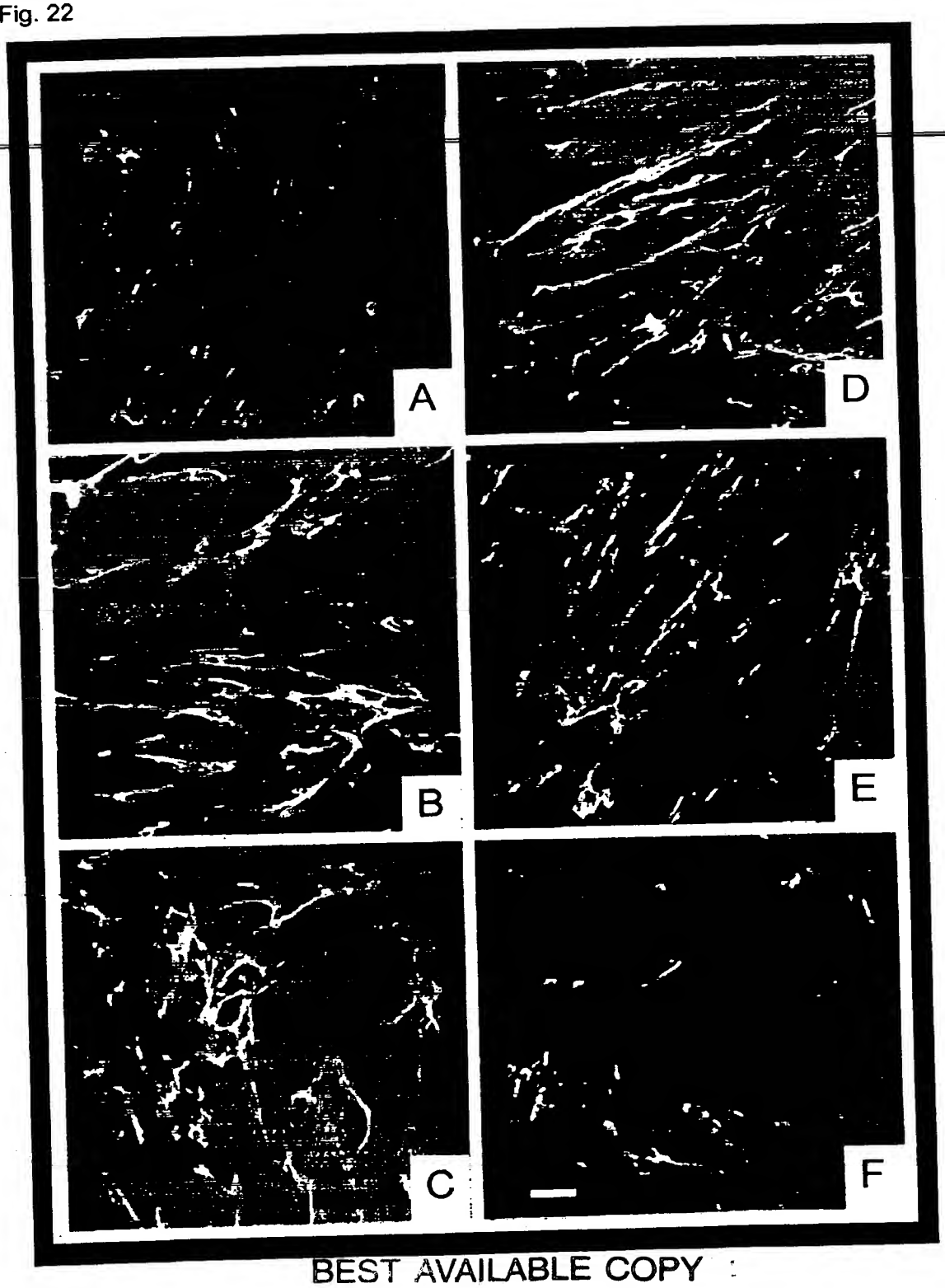
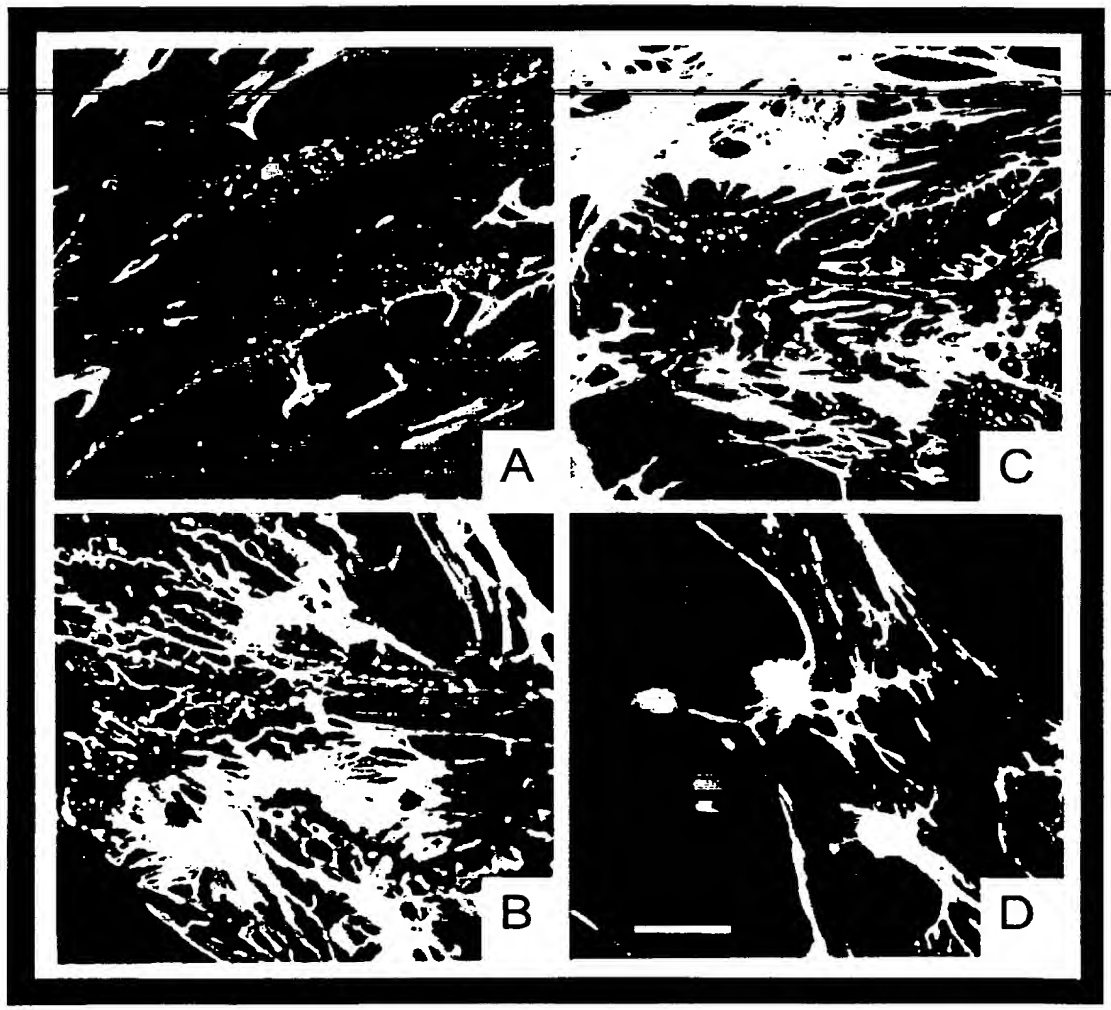


Fig. 23



Claims

1. Material having a first contact angle and comprising a substratum having a second contact angle, said substratum being contacted by a macromolecule, wherein the relation between said first and second contact angle as defined by the ratio between
   i) the difference between said second contact angle, when no macromolecule is present, and said first contact angle, and
   ii) the difference between said second contact angle, when no macromolecule is present, and the contact angle of said substratum, when said substratum is saturated by said macromolecules as defined herein,

   is more than -0.6 and less than 0.6.

2. Material having a first contact angle and comprising a substratum having a second contact angle, said substratum being contacted by a plurality of soluble substances capable of forming a self-assembled monolayer comprising a macromolecule and having a third contact angle, wherein the relation between said contact angles as defined by the ratio between
   i) the difference between the third contact angle of said monolayer, when no macromolecule is present, and said first contact angle, and
   ii) the difference between the third contact angle of said monolayer, when no macromolecule is present, and the contact angle of said self-assembled monolayer, when said monolayer is saturated by said macromolecules as defined herein,

   is more than -0.6 and less than 0.6.

3. Material according to claim 2, wherein said soluble substance is selected from the group consisting of molecules capable of forming a self-assembled monolayer.

4. Material according to any of claims 1 to 3, wherein said substratum is pretreated or modified.

5. Material according to claim 4 wherein said pretreated or modified substratum is the result of said substratum being contacted by and/or operably linked to a charged group or a hydrophilic compound.

6. Material according to any of the preceding claims, wherein said first contact angle is the advancing contact angle.

7. Material according to claim 6, wherein said first contact angle is in the range of from 50 degrees to 140 degrees.

8. Material according to claim 6, wherein said first contact angle is in the range of from 60 degrees to 125 degrees.

9. Material according to claim 6, wherein said first contact angle is in the range of from 70 degrees to 120 degrees.

10. Material according to claim 6, wherein said first contact angle is in the range of from 75 degrees to 110 degrees.

11. Material according to claim 6, wherein said first contact angle is in the range of from 80 degrees to 100 degrees.

12. Material according to claim 6, wherein said ratio is less than 0.50.

13. Material according to claim 6, wherein said ratio is less than 0.40.

14. Material according to claim 6, wherein said ratio is less than 0.30.

15. Material according to claim 6, wherein said ratio is less than 0.25.

16. Material according to claim 6, wherein said ratio is less than 0.20.

17. Material according to claim 6, wherein said ratio is less than 0.15.

18. Material according to claim 6, wherein said ratio is less than 0.10.

19. Material according to claim 6, wherein said ratio is less than 0.05.

20. Material according to any of claims 1 to 5, wherein said first contact angle is the receding contact angle and wherein said ratio is less than 0.40.

21. Material according to claim 20, wherein said first contact angle is in the range of from 30 degrees to 120 degrees.

22. Material according to claim 20, wherein said first contact angle is in the range of from 40 degrees to 110 degrees.

23. Material according to claim 20, wherein said first contact angle is in the range of from 50 degrees to 100 degrees.

24. Material according to claim 20, wherein said first contact angle is in the range of from 60 degrees to 90 degrees.

25. Material according to claim 20, wherein said first contact angle is in the range of from 70 degrees to 80 degrees.

26. Material according to claim 20, wherein said ratio is less than 0.35.

27. Material according to claim 20, wherein said ratio is less than 0.30.

28. Material according to claim 20, wherein said ratio is less than 0.25.

29. Material according to claim 20, wherein said ratio is less than 0.20.

30. Material according to claim 20, wherein said ratio is less than 0.15.

31. Material according to claim 20, wherein said ratio is less than 0.10.

32. Material according to claim 20, wherein said ratio is less than 0.05.

33. Material according to any of the preceding claims, wherein said material, when contacted by a first determinant comprising a compound selected from the group consisting of a polypeptide, or part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof, is capable of maintaining said compound in a biologically active form.

34. Material according to claim 33 wherein said compound is a polypeptide or part thereof.

35. Material according to claim 33 or 34 further comprising said first determinant comprising said compound, wherein said first determinant is maintained in a biologically active form when contacted by said substratum and/or said macromolecule.

36. Material according to claim 35 wherein said biologically active form is essentially a biologically active conformation.

37. Material according to any of claims 33 to 36 wherein said biologically active form or conformation is maintained and/or improved and/or stabilized by means of the cooperactivity of said substratum and said macromolecule.

38. Material according to claim 33 to 37 wherein said biologically active form or confirmation is maintained and/or improved and/or stabilized when contacted by said substratum and said macromolecule.

39. Material according to any of the preceding claims, wherein said material is biocompatible.

40. Material according to any of the preceding claims, wherein the weight increase per area unit arising from the part of the macromolecule essentially consisting of poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO) is less than
2.0×10^{-22} grams (g) per square nanometer (nm^2).

41. Material according to claim 40, wherein said difference is less than 1.0×10^{-22} grams (g) per square nanometer (nm^2).

42. Material according to claim 40, wherein said difference is less than 0.8×10^{-22} grams (g) per square nanometer (nm^2).

43. Material according to claim 40, wherein said difference is less than 0.5×10^{-22} grams (g) per square nanometer (nm^2).

44. Material according to claim 40, wherein said difference is less than 0.3×10^{-22} grams (g) per square nanometer (nm^2).

45. Material according to any of the preceding claims, wherein said substratum is contacted by a plurality of soluble compounds capable of forming a layer of self-assembled macromolecules.

46. Material according to claim 45, wherein said soluble compounds are n-alkane chains preferably containing from 8 to 24 carbons.

47. Material according to any of the preceding claims wherein each macromolecule is associated with an excluded volume.

48. Material according to any of the preceding claims, wherein said substratum comprises a hydrophobic polymer.

49. Material according to claim 48, wherein said substratum is at least substantially flexible.

50. Material according to claim 48, wherein said substratum is a film.

51. Material according to claim 45, wherein said substratum is essentially rigid or at least substantially non-flexible.

52. Material according to claim 51, wherein said substratum comprises a crystalline structure capable of supporting a self-assembled monolayer such as gold, silicon oxide, and similar crystalline structures and/or structures that are smooth on a nanometer scale.

53. Material according to any of the preceding claims, wherein said macromolecule comprises a hydrophilic polymer.

54. Material according to claim 53, wherein said macromolecule comprises an amphiphilic polymer.

55. Material according to any of the preceding claims, wherein said macromolecule has a molecular weight (MW) of more than 400 Dalton (Da).

56. Material according to claim 55, wherein said macromolecule has a molecular weight (MW) of more than 1.000 Dalton (Da).

57. Material according to claim 55, wherein said macromolecule has a molecular weight (MW) of more than 5.000 Dalton (kDa).

58. Material according to claim 55, wherein said macromolecule has a molecular weight (MW) of more than 10.000 Dalton (Da).

59. Material according to claim 55, wherein said macromolecule has a molecular weight (MW) of more than 50.000 Dalton (Da).

60. Material according to claim 55, wherein said macromolecule has a molecular weight (MW) of more than 100.000 Dalton (Da).

61. Material according to any of the preceding claims, wherein said macromolecule is a conjugate comprising a head group, a guiding group, a linker group, a polymer chain or a main body, and a functional end group.

62. Material according to claim 61, wherein said head group is capable of forming a chemical bond.

63. Material according to claim 61, wherein said head group may adsorb to the substratum.

64. Material according to claim 61, wherein said head group is capable of forming an ionic bond.

65. Material according to claim 61, wherein said head group may be entangled into or with the substratum.

66. Material according to claim 61, wherein said head group is capable of forming a self-assembled monolayer.

67. Material according to claim 61, wherein said guiding group is a bifunctional group comprising an aliphatic, linear or weakly branhed group.

68. Material according to claim 61, wherein said linker group is capable of being enzymatically or chemically hydrolysed.

69. Material according to claim 61, wherein said linker group is hydrolytically unstable.

70. Material according to claim 61, wherein said linker group is essentially stable against cleavage under practical circumstances.

71. Material according to claim 61, wherein said polymer chain or main body is preferably hydrophilic, uncoiling in an aqueous environment and exhibiting an excluded volume.

72. Material according to claim 64, wherein said functional end group is capable of linking permanently or reversibly other biological or synthetic molecules or materials.

73. Material according to any of claims 33 to 72, wherein said first determinant comprises a biologically active compound comprising a polypeptide, or a part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof.

74. Material according to claim 73, wherein said biologically active compound comprises a polypeptide.

75. Material according to claim 73, wherein said biologically active compound is selected from the group consisting of membrane associated and/or extracellular matrix polypeptides natively produced by a microbial cell, a plant cell or a mammalian cell.

76. Material according to claim 73 wherein said biologically active compound is selected from the group consisting of a polypeptide, an antibody, a polyclonal antibody, a monoclonal antobody, an immunogenic determinant, an antigenic determinant, a receptor, a receptor binding protein, an interleukine, a cytokine, a cellular differentiation factor, a cellular growth factor, and an antagonist to a receptor.

77. Material according to claim 73, wherein said biologically active compound is a synthetic polypeptide, or part thereof, capable of contacting said substratum and/or said macromolecule.

78. Material according to claim 73, wherein said biologically active compound is a synthetic polypeptide, or part thereof, capable of contacting said substratum and said macromolecule.

79. Material according to claim 73, wherein said biologically active compound is an adhesion polypeptide, preferably fibronectin or vitronectin.

80. Material according to any of claims 33 to 79, wherein said biologically active compound results in an improved contact between said material and a biological entity, such as a biological cell or a virus, or part thereof, including a polypeptide, or a part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof.

81. Material according to any of the preceding claims, said material further comprising a second determinant.

82. Material according to claim 81, wherein said second determinant comprises a biological entity, such as a biological cell or a virus, or part thereof, including a polypeptide, or a part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof.

83. Material according to claim 81, wherein said biological entity is selected from the group consisting of a polypeptide, an antibody, a polyclonal antibody, a monoclonal antobody, an immunogenic determinant, an antigenic determinant, a receptor, a receptor binding protein, an interleukine, a cytokine, a differentiation factor, a growth factor, and an antagonist to the receptor.

84. Material according to claim 82, wherein said biological cell, or part thereof, is selected from the group consisting of a mammalian cell, including a human cell and an animal cell, a plant cell, a microbial cell, including a eukaryotic microbial cell, including a yeast and a fungus, and a prokaryotic microbial cell including a bacteria.

85. Material according to claim 84 wherein said biological cell is a mammalian cell.

86. Material according to claim 82, wherein said virus, or part thereof, is selected from a mammalian virus, including a human virus and an animal virus, a plant virus, a microbial virus, including a eukaryotic microbial virus, including a yeast virus and a fungal virus, and a prokaryotic microbial virus including a bacteriophage.

87. Material according to claim 86 wherein said virus is a mammalian virus.

88. Material according to any of the preceding claims, wherein said substratum is porous and preferably a membrane.

89. Material according to claim 88, wherein the flux of water through said material is substantially unchanged as compared to the flux of water through said porous substratum.

90. Material according to any of claims 1 to 89, wherein said substratum is non-porous and/or substantially non-penetrable to water.

91. Material according to any of the preceding claims for use in a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation ex vivo.

92. Material according to any of the preceding claims for use in a method of separating and/or isolating biological material ex vivo.

93. Material according to any of the preceding claims for use in a method of producing a biohybrid organ ex vivo.

94. Material according to any of claims 1 to 93 for use in a diagnostic method carried out on the human or animal body.

95. Material according to any of claims 1 to 94 for use in a method of therapy carried out on the human or animal body.

96. Material according to any of claims 1 to 95 for use in a method of surgery carried out on the human or animal body.

97. Material according to any of claims 1 to 96 for use in a method of producing a biohybrid organ in vivo.

98. Material according to any of claims 1 to 97 for use as a carrier for in vivo delivery of a medicament to a human or animal body in need of said medicament.

99. Material according to any of claims 1 to 98 for use in a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation in vivo.

100. Material according to any of claims 1 to 99 for use in a method of separating and/or isolating biological material in vivo.

101. Composition comprising the material according to any of the preceding claims and a physiologically acceptable carrier.

102. Pharmaceutical composition comprising the material according to any of claims 1 to 100 or the composition of claim 101 and a pharmaceutically active ingredient and optionally a pharmaceutically active carrier.

103. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a method of therapy carried out on the human or animal body.

104. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a method of surgery carried out on the human or animal body.

105. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a diagnostic method carried out on the human or animal body.

106. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a method of producing a biohybrid organ in vivo.

107. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 as a carrier for in vivo delivery of a medicament to a human or animal body in need of said medicament.

108. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation in vivo.

109. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a method of separating and/or isolating biological material in vivo.

110. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation ex vivo.

111. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a method of separating and/or isolating biological material ex vivo.

112. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in a method of producing a biohybrid organ ex vivo.

113. Use of the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 in the manufacture of an implantable organ or part thereof.

114. Use of the material according to any of claims 1 to 100 as a carrier for a pharmaceutically active ingredient or a pharmaceutical composition.

115. Method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation ex vivo, said method comprising the steps of contacting a cell with the material according to any of claims 1 to 100 or the composition

according to claim 101 or the pharmacetical composition according to claim 102 and incubating said cell and said material under conditions allowing said cell to grow and/or proliferate and/or differentiate.

116. Method of separating and/or isolating biological material ex vivo, said method comprising the steps of contacting said biological material to be separated and/or isolated with the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 and incubating said biological material and said material under conditions that allow separation and/or isolation.

117. Method of producing a biohybrid organ ex vivo, said method comprising the stepsof contacting biohydrid organ cells with the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 and incubating said biohybrid organ cells under conditions allowing the production of said biohybrid organ.

118. Method of therapy carried out on the human or animal body, said method comprising the step of contacting said body with the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102.

119. Method of surgery carried out on the human or animal body, said method comprising the step of contacting said body with the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102.

120. Method of diagnosis carried out on the human or animal body, said method comprising the steps of contacting said body with the material according to any of claims 1 to 100 or the composition according to claim 101 or the

pharmacetical composition according to claim 102 and detecting a signal generated directly or indirectly by said material.

121. Method of controlling cellular growth and/or cellular proliferation and/or cellular differentiation in vivo, said method comprising the steps of contacting a cell with the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 and incubating said cell and said material under conditions allowing said cell to grow and/or proliferate and/or differentiate.

122. Method of separating and/or isolating biological material in vivo, said method comprising the steps of contacting said biological material to be separated and/or isolated with the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 and incubating said biological material and said material under conditions that allow separation and/or isolation.

123. Method of producing a biohybrid organ in vivo, said method comprising the steps of contacting biohydrid organ cells with the material according to any of claims 1 to 100 or the composition according to claim 101 or the pharmacetical composition according to claim 102 and incubating said biohybrid organ cells under conditions allowing the production of said biohybrid organ.

124. Method of in vivo delivery of a medicament to a human or animal body in need of said medicament, said method comprising the steps of contacting said body with the pharmacetical composition according to claim 102 and incubating said body contacted by said pharmaceutical composition under conditions allowing delivery of said medicament.

125. Method for producing the material according to any of claims 1 to 100, said method comprising the steps of

i) providing a substratum having a second contact angle, and

ii) contacting said substratum with a composition comprising a plurality of macromolecules.

126. Method according to claim 125, wherein said substratum comprises a hydrophobic polymer.

127. Method according to claim 125, wherein said substratum is pretreated prior to being contacted by said macromolecule.

128. Method according to claim 127, wherein said pretreatment is effective in increasing the wettability of said substratum.

129. Method according to claim 125, wherein said macromolecule comprises a hydrophilic polymer.

130. Method according to claim 125, wherein said macromolecule comprises a latently reactivatable polymer.

131. Method according to claim 125, wherein macromolecule has a molecular weight (MW) of more than 400 Dalton (Da).

132. Method according to claim 125, wherein said macromolecule comprises a conjugate comprising a cross linkable head group, a linker group, a polymer chain, and a functional end group.

133. Method according to claim 132, wherein said cross linkable head group is a photo-reactivatable aryl azide head group.

134. Method according to claim 132, wherein said macromolecule further comprises a modifying agent.

135. Method according to claim 134 wherein said modifying agent is capable of contacting said substratum and forming a self assembled monolayer.

136. Method according to any of claims 125 to 135 for producing the material according to any of claims 1 to 100, said method comprising the further step of contacting said material with a first determinant comprising a biologically active compound.

137. Method according to claim 136, wherein said biologically active compound is selected from the group consisting of a polypeptide, an antibody, a polyclonal antibody, a monoclonal antobody, an immunogenic determinant, an antigenic determinant, a receptor, a receptor binding protein, an interleukine, a cytokine, a cellular differentiation factor, a cellular growth factor, and an antagonist to a receptor.

138. Method according to claim 136, wherein said biologically active compound is a membrane associated and/or extracellular matrix polypeptide natively produced by a microbial cell, a plant cell or a mammalian cell.

139. Method according to any of claims 136 to 138 for producing the material according to any of claims 1 to 100, said method comprising the further step of contacting said material with a second determinant comprising a biological entity.

140. Method according to claim 139, wherein said biological entity comprises a cell or a virus, or a part thereof.

141. Method according to claim 140, wherein said cell, or part thereof, is selected from the group consisting of a mammalian cell, including a human cell and an animal cell, a plant cell, a microbial cell, including a eukaryotic microbial cell, including a yeast and a fungus, and a prokaryotic microbial cell including a bacteria.

142. Method according to claim 140, wherein said virus, or part thereof, is selected from a mammalian virus, including a human virus and an animal virus, a plant virus, a microbial virus, including a eukaryotic microbial virus, including a yeast virus and a fungal virus, and a prokaryotic microbial virus including a bacteriophage.

143. Method according to claim 139, wherein said biological entity comprises a polypeptide, or a part thereof, a nucleic acid moiety, a carbohydrate moiety, and a lipid moiety, including any combination thereof.

144. Method according to claim 139, wherein said biological entity is selected from the group consisting of a polypeptide, an antibody, a polyclonal antibody, a monoclonal antobody, an immunogenic determinant, an antigenic determinant, a receptor, a receptor binding protein, an interleukine, a cytokine, a differentiation factor, a growth factor, and an antagonist to the receptor.

Abstact

The present invention is in the area of biomaterials, i.e. materials that are used in contact with living tissue and biological fluids for prosthetical, therapeutical, storage and the like. In particular, the invention relates to a novel approach of creating biocompatible surfaces, said surfaces being capable of functionally interacting with biological material. Said biocompatible surfaces comprise at least two components, such as a hydrophobic substratum and a macromolecule of hydrophilic nature, that cooperactively form a novel biocompatible surface. The novel approach is based on contacting said hydrophobic substratum with a laterally patterned monomolecular layer of hydrophilic and flexible macromolecules that exhibit a pronounced excluded volume. The surface is, in respect to polarity and morphology, a molecularly heterogenous surface. Structural features of said macromolecular monolayer (as e.g. the layer thickness or its lateral density) are determined by, i) the structural features of the layer forming macromolecules (as e.g. their molecular weight or their molecular architecture) and, ii) the method of creating said monomolecular layer (as e.g. by physi- or chemisorbing, or by chemically binding said macromolecules). The structural features of the layer forming macromolecule(s) is in turn determined by synthesis.